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AGRICULTURAL IRRIGATION DRAINWATER STUDIES

in support of the

SAN JOAQUIN VALLEY DRAINAGE PROGRAM

FINAL REPORT

U.S. DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
NATIONAL FISHERIES CONTAMINANT RESEARCH CENTER
ROUTE 2, COLUMBIA, MISSOURI 65201

AUGUST 1990

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Project Coordinator: A. Dennis Lemly, Ph.D.

funded through
INTRA-AGENCY AGREEMENT 6-AA-20-04170
U.S. BUREAU OF RECLAMATION
U.S. FISH AND WILDLIFE SERVICE

AUGUST 1990

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EXECUTIVE SUMMARY

The National Fisheries Contaminant Research Center recently completed a four year program of studies to identify and characterize the toxicity of contaminants associated with agricultural irrigation drainwater in the Central Valley of California. The research effort was divided into three major program areas: 1) monitoring of water quality and contaminant levels in biota to identify contaminants of concern and assess field-level exposure, 2) toxicological asessment of drainwater contaminants using fish and invertebrates in a battery of laboratory and on-site tests, and 3) field and laboratory bioaccumulation studies to evaluate the role of the food chain in the transport, chemical speciation, and biological cycling of contaminants. The results of these studies provide critical information regarding the hazard of irrigation drainage to aquatic life in California and other western states, as well as the validity of current and proposed water quality criteria for specific irrigation drainwater contaminants.

Water Quality Monitoring and Contaminants in Biota

Field surveys of water quality and contaminant levels were made during the fall (September-December) and spring (February-May) in twenty-seven locations encompassing the San Joaquin and Sacramento Rivers, and their tributaries. Results of these surveys indicated that as many as seven chemical constituents were of concern (selenium, boron, molybdenum, chromium, copper, mercury, zinc). However, due to funding limitations, selenium, boron, and molybdenum were given priority in the detailed surveys of water quality and food-chain residues. Selenium was markedly elevated in the water (5-10 ug/l) and aquatic organisms and detritus (5-23 ug/g dry weight) at three sites: Salt and Mud (north) Sloughs and the San Joaquin River upstream of Hills Ferry Road. Selenium concentrations in biota from these sites equalled or exceeded toxic dietary concentrations for selenium-sensitive species such as bluegill and largemouth bass (5-15 ug/g dry weight). These findings illustrate the importance of the food chain in selenium cycling and bioaccumulation. Boron, molybdenum, and selenium in water were readily bioconcentrated by filamentous algae, which provides the first link in the bioaccumulation phenomenon. Selenium concentrations increased up the food chain in a pattern suggestive of biomagnification.

Concentrations of contaminants in fish tissues indicated that selenium was of primary concern followed by molybdenum, boron, chromium, and mercury. Selenium was markedly elevated at three sites: Salt and Mud (north) Sloughs and the San Joaquin River upstream of Hills Ferry Road. In these locations the tissue levels of selenium were sufficiently elevated (7-11 ug/g dry weight) to indicate that reproductive impairment may be imminent or already occurring in the fish population. Moreover, selenium levels varied in a recognizable pattern that corresponded to existing and historical inputs of tile drainwater into the San Joaquin River and its tributaries. Although the concentrations of molybdenum, boron, chromium, and mercury were occasionally elevated as compared to baseline concentrations in the National Contaminant Biomonitoring Program of the U.S. Fish and Wildlife Service, no clear toxicological implications were found. For these trace elements, monitoring is recommended to further document trends in contaminant levels and identify possible impacts.

Further field studies are needed to fully determine the environmental hazard of discharging agricultural drainwater into the San Joaquin River and its tributaries. Such studies should include the following:

1) Factors controlling the uptake and excretion of boron and molybdenum in aquatic biota should be determined. Although these elements apparently do not biomagnify through the food chain, they are bioaccumulated in the primary producers (algae) and thus pose a toxic threat to animals that forage on the lower trophic levels. Presently, the toxic thresholds for these elements to fish are unknown.

2) The interactions and possible synergistic effects of elevated dietary and waterborne boron, selenium, and molybdenum on aquatic biota needs further clarification. Past studies have typically investigated only one trace element at a time and the effects of this contaminant mixture are still poorly understood.

3) The mechanisms that allow unusually high concentrations of selenium to accumulate in particulate organic detritus must be better understood. Detritus is a significant link in many aquatic food chains and it may account for a large part of the selenium residues occurring in fish from certain locations.

4) Based on general guidelines for toxic thresholds of selenium in water, sediment, food organisms, and fish tissues, the concentrations of this element are sufficiently elevated to impair the reproduction of centrarchids in at least three localities in the San Joaquin River system. Studies that assess the health and reproductive success of local fish populations are needed in order to field validate these toxicity thresholds and further determine the environmental damage caused by seleniferous agricultural drainwater in the San Joaquin River.

Toxicological Assessment of Drainwater Contaminants

Studies with invertebrates: Acute (48-hr) and chronic (21 or 30 days) waterborne exposures were used to assess the toxicity of irrigation drainwater contaminants to aquatic invertebrates. Because of funding limitations, the only drainwater contaminant tested was selenium, using the selenate, selenite, and seleno-L-methionine forms. Daphnids (crustaceans) were much more sensitive (about 3-4 times more sensitive to selenate and selenite; about 1000 times more sensitive to seleno-L-methionine) to this trace element than midges (insects) and for both organisms, the relative toxicity of different selenium forms was: seleno-L-methionine > > > selenite > selenate. Selenate and selenite were toxic to invertebrate reproduction (adults were not harmed) at levels of 300-1000 ug/l, however, as little as 4 ug/l seleno-L-methionine was sufficient to kill adult daphnids within 48 hours. With this level of toxicity, the geometric mean maximum acceptable concentration for selenomethionine would be about 0.16 ug se/l, a concentration that is scarcely detectable with standard analytical chemistry methods. These studies illustrate the marked difference in species sensitivity to selenium and point to the need to assess environmental hazard using organisms from different trophic levels and different taxonomic status within trophic levels.

Comparative studies using different chemical forms of selenium are crutial to our understanding of selenium cycling and toxicity in aquatic habitats. These invertebrate studies have identified the need for additional investigations that will:

1) Determine the biological basis for the dramatic difference in sensitivity to organic selenium between species of invertebrates.

2) Evaluate the latest EPA Continuous Criterion Concentration (5 ug/l total Se) in light of the extreme toxicity of organic selenium. Additional data are needed on the occurrence, chronic toxicity, and fate of organic selenium compounds in fresh water before an environmentally sound hazard evaluation can be made.

Studies with fish: The waterborne toxicity of irrigation drainwater contaminants to fish was evaluated with early life-stage exposures using striped bass, chinook salmon, and bluegill. These studies focused on selenium (selenate, selenite, seleno-L-methionine) but in the case of chinook salmon, also included boron, molybdenum, arsenic, cadmium, copper, chromium, mercury, vanadium, nickel, silver, manganese, and zinc. The toxicity of combined dietary and waterborne selenium was assessed using juvenile chinook salmon and juvenile and adult bluegill. An in-depth evaluation of the effects of dietary and waterborne selenium on chinook salmon physiology and smoltification was made. A long-term combined dietary-waterborne study was conducted with bluegill to determine the effect of selenium on reproductive success. Field studies were conducted in the San Joaquin Valley to determine the toxicity of actual tile drainwater to juvenile chinook salmon and striped bass.

A) Chinook Salmon: Waterborne exposures were conducted with selenate and selenite, however, no studies were conducted with selenomethionine. The swimup-fry life stage was 3-10 times more sensitive than were the eyed-egg or alevin life stages. Both selenate and selenite were toxic in the mg/l range (96-hr LC50's = 10-300+ mg/l, 120 day toxic level > 139 ug/l). The potential hazard of these forms of selenium to indigenous salmon populations in California was estimated using a standard method that compares the 96-hour LC50 values with the expected environmental concentrations -- i.e., in effluent from the San Luis Drain. Findings suggest that inorganic waterborne selenium does not pose a direct toxic threat to this fish species. However, the implications of organic selenium in this assessment are unknown. Moreover, bioaccumulation and resulting dietary toxicity are not considered in this type of assessment. It is thus very important to consider all routes of exposure and all chemical forms of selenium when making an environmental hazard evaluation for drainwater contaminants.

A similar hazard assessment was made for the waterborne exposures with cadmium, copper, mercury, zinc, vanadium, arsenic, chromium, boron, and molybdenum. When tested in single element exposures, four of the nine trace elements (boron, cadmium, copper, and zinc) has LC50 values that were less that a factor of 100 of their concentrations in undiluted San Luis Drain water, suggesting that there is a high potential for direct toxicity to juvenile chinook salmon in receiving waters. In contaminant mixture studies that utilized 13 trace elements, with and without selenium, the toxicities were additive rather than antagonistic or synergistic. The joint toxicites seen in the mixture studies yielded margins of safety that were very small (5-6), indicating an extremely high potential for direct waterborne toxicity to young salmon in the environment. The toxicities, margins of safety, and potential hazard of these trace elements was essentially the same in brackish water and fresh water.

Feeding studies revealed that selenium was the major toxic component in a field-source (natural) diet, however, boron, chromium, and strontium may have also contributed to the observed toxicity. Synthetic diets formulated with selenomethionine generated toxicity profiles that closely matched the toxicity of the field-source diets. This information suggests that a large part of the selenium present in food-chain organisms in the environment may exist as selenomethionine. When salmon were fed selenomethionine permeated diets, effects began to occur at or near a concentration of 5 ug/g dry weight.

The most subtle effect appeared to be reduced growth (length and weight) followed closely by gill enzyme activity, ion regulation, smoltification success, and survival. Reduced survival in the selenomethionine studies began to occur when the diet contained 9 ug/g Se dry weight. Residue levels in fish tissues began to increase when dietary concentrations reached 3 ug/g Se, and were present at 5-8 ug/g Se (whole body basis) when mortality began to occur. The young-fry life stage appeared to be more sensitive than advanced fry, and also accumulated greater selenium residues. In combined waterborne-dietary exposures, the residue concentrations and toxicity were attributed primarily to dietary selenium. The importance of the food chain in selenium toxicity is strongly supported by these findings.

The field toxicity studies indicated that full-strength (undiluted) tile drainwater can kill fingerling chinook salmon within 28 days. The toxicity was partly attributable to an unusual ionic composition (unusual amounts and ratios of chlorides, sulfates, and sodium) in addition to the direct toxicity of trace elements. High salinity did not contribute to the overall toxicity of drainwater because fish held in reconstituted seawater did not experience excessive mortality or exhibit reduced growth and body condition. Of the trace elements measured (boron, chromium, copper, molybdenum, and selenium), only selenium exceeded the EPA national criterion for protecting aquatic life (concentrations exceeded 100 ug/l). However, only boron accumulated to excessive levels in the tissues of fish held in full-strength drainwater (concentrations exceeded 130 ug/g dry weight after 28 days).

To better understand the potential toxicity of tile drainage water to chinook salmon, additional research is needed as follows:

1) Definitive waterborne exposures of larval and fingerling chinook salmon to selenomethionine need to be conducted to assess the importance of organoselenium in toxicity.

2) Definitive waterborne exposures of larval and fingerling salmon to molybdenum should be conducted to assess the environmental hazard of this trace element.

3) The chronic toxicity of sodium sulfate (the predominant "salt" occurring in tile drainage water) should be characterized. Although the State of California has established water quality objectives for boron, molybdenum, selenium, and conductivity (a measure of the concentration of total dissolved solids) in the San Joaquin River and some tributaries, there are no objectives for sodium sulfate. It is known that some freshwater fishes cannot tolerate concentrations of sodium sulfate greater than 100 mg/l.

4) The environmental factors influencing the accumulation of boron in fish must be further investigated. Chinook salmon held for 28 days in full-strength drainwater accumulated over 130 ug/g of boron, which is considerably higher than published values for whole freshwater fish. Because there is no known nutritional requirement for boron in animals, excessive accumulations of this trace element in tissues could represent the initial onset of toxicity.

B) Striped Bass: Because of funding limitations, only selenium was examined in waterborne exposure studies. No dietary or combination dietary-waterborne exposures were conducted. Exposure of larval striped bass to selenate, selenite, and seleno-L-methionine revealed that these chemical forms are markedly different in their toxicity. Both selenate and selenite were toxic at mg/l levels (96-hr LC50 = 1-40 mg/l, 90 day toxic level > 3.0 mg/l) but selenomethionine was toxic at extremely low concentrations (96-hr LC50 = 0.003 mg/l). Because selenomethionine was very toxic to striped bass in 96-hr exposures, and the cost of long-term tests with selenomethionine is quite high, chronic studies (90 days) were not possible. The lowest effect level for this chemical form of selenium is probably well below 1 ug/l. These findings have important implications for the hazard assessment of irrigation drainage because previous studies have documented large concentrations (several ug/l) of organic selenium in the waters that existed at Kesterson Reservoir. Dissolved organic selenium probably occurs in many other locations as well. If even a small fraction of the total waterborne selenium is present in a seleno-amino form, there is a high potential for toxicity to striped bass.

Field studies showed that full-strength tile drainwater can kill 100% of fingerling striped bass within 23 days. The mortality exhibited a dose-response pattern and when drainwater was diluted with freshwater, more subtle effects (reduced growth and weight gain) were seen. High salinity was not a factor contributing to toxicity; a combination of elevated trace elements and unusual ionic composition was the primary cause. Although selenium levels were markedly elevated in the drainwater (greater than 100 ug/l), only boron accumulated in striped bass tissues, indicating that the food chain is the primary route of uptake leading to selenium bioaccumulation.

Several aspects of the toxicity of irrigation drainage to striped bass warrant additional study, including the following:

1) No dietary exposures were conducted in these studies. Both dietary and combined dietary-waterborne exposures should be used to further assess the hazard of selenium to early life stages of striped bass.

2) The occurrence and concentration of amino forms of selenium in the environment should be determined. Without this information it is impossible to interpret the significance of the extreme toxicity of selenomethionine.

3) Apparent differences in species sensitivity to selenium should be examined and further evaluated. The studies conducted with striped bass showed that inorganic selenium was relatively non-toxic at waterborne levels up to about 3.0 mg/l, however, this is not true

for other species. For example, mortality occured in bluegill at concentrations less than 1 mg/l. The difference in selenium tolerance may be species specific or it may be related to water quality variables such as salinity or hardness.

4) As with chinook salmon, the chronic toxicity of sodium sulfate should be determined in order to better interpret the significance of ionic composition in the toxicity profile of drainwater. Also, the toxicological significance of elevated boron in striped bass tissues should be determined.

C) Bluegill: Waterborne and dietary studies were conducted to assess the effects of selenium on non-migratory freshwater fish in the San Joaquin Valley using bluegill as the experimental model. Because of the complexity of the anadromous life cycle of striped bass and chinook salmon, bluegill were also used in long-term studies to evaluate the effects of selenium on reproductive success. In short-term waterborne exposures, the selenate and selenite forms of selenium were toxic to fingerling bluegill in the mg/l range (96-hr LC50 = 7-120 mg/l, 60 day no-effect level = 0.34-0.68 mg/l). However, seleno-L-methionine was much more toxic, with 96-hr LC50's of 7-16 ug/l. Because of it's extreme acute toxicity, and prohibitively high cost, definitive chronic waterborne exposures using selenomethionine were not conducted. However, as with striped bass, the lowest effect level for selenomethionine to bluegill is probably below 1 ug/l, indicating a potential hazard when dissolved organic selenium is present in the environment. Dietary studies with fingerling bluegill indicated that mortality began to occur when concentrations were 6.5 mg/kg dry weight or greater. The corresponding whole-body tissue residues accumulated in these fish ranged from 5-10 mg/kg dry weight.

In the reproduction study, no effects were seen on the adult fish even in the highest treatments (33 mg/kg selenomethionine in the diet and 10 ug/l selenite in the water).

However, the influence of adult exposure to dietary selenium on the survival of fry was the most significant biological effect observed in the study. Fecundity and hatchability of eggs were not affected at any treatment level but fry resulting from parents in the high exposures (33 mg/kg dry weight diet; 10 ug/l water) consistently failed to survive. These fish exhibited almost total mortality prior to the critical period in their development when they switch from internal (yolk sac) feeding to external food sources. The threshold for these effects occurs at dietary concentrations between 16 mg/kg (no effect) and 33 mg/kg dry weight (complete mortality). Tissue residues in the adult fish exposed to the 33 mg/kg dietary levels were about 16-18 mg/kg dry weight in whole body analyses, 35-40 ug/g in ovaries, and 30-35 mg/kg in testes. One of the most striking and biologically important findings of these studies is that selenium may not elicit overt toxicological responses in adult fish even though reproductive success is completely impaired. These results lend support to field studies that have documented high selenium residues in fish populations and a gradual loss or complete absence of young-of-the-year fish. In addition to documenting reproductive effects, this study provides data on tissue residues that may provide a tool for making environmental assessments of selenium exposure and potential toxicity in fish populations.

Although these studies identified important factors in selenium toxicity to fish, several equally important factors remain to be studied. Some of these are:

1) Elucidation of selenomethionine toxicity -- the extreme toxicity of this chemical form of selenium to fish suggests that a high potential environmental hazard exists. However, it is not clear whether the toxicity is due exclusively to the parent compound (selenomethionine) or some combined toxicity of the parent compound and breakdown products (e.g. methyl selenides, selenonium ion, etc). Chemical speciation studies

performed during aqueous exposure of fish to selenomethionine could serve to identify specific, and perhaps additional, compounds responsible for the observed toxicity.

2) Potential ameliorative effect of sulfate on selenium toxicity -- some studies suggest that, due to the chemical similarity of selenium and sulfur, the presence of sulfates in water may reduce the toxicity of inorganic selenium, particularly selenate, through competitive uptake. Information from other drainwater studies in this report indicates that variable sulfate levels do not affect the bioaccumulation and distribution of selenium, but the potential mediation of toxicity was not assessed. Studies conducted with fish exposed to waterborne selenium concentrations near the toxic threshold in conjunction with various levels of sulfate could demonstrate whether such an interaction occurs.

3) Expansion of the dietary selenium exposure range between 15 and 33 mg/kg -- the results of the bluegill reproductive study indicate that a threshold dietary effect concentration exists between 15 and 33 mg/kg dry weight, beyond which fry survival is severely diminished. However, this study did not examine intermediate concentrations. Expansion of the selenium exposure concentration range (e.g. 15, 20, 25, 30, 35 mg/kg) would provide a more precise estimate of the lowest dietary effect level. Exposing bluegill to a series of dietary selenium concentrations within this range would also identify whether there is a dose-response, as there is for waterborne inorganic selenium, or a sharp toxicity threshold.

Bioaccumulation and Chemical Speciation Studies

These laboratory studies were conducted to determine relationships between selenium residues in fish, selenium concentrations and speciation in water, and selenium dynamics in aquatic food chains. Two types of studies were conducted; microcosm selenium fate studies, which evaluated factors affecting the formation and persistence of selenium species in aquatic ecosystems; and food-chain selenium bioaccumulation studies, which compared the bioconcentration of selenium species from water and the transfer of accumulated residues to consumers in a simple food chain (algae, invertebrates, fish).

The distribution and bioaccumulation of selenium in microcosms varied depending on the types of sediments used and the selenium species. A mixture of selenium forms (1:100:1000 as selenomethionine, selenite, selenate) was sorbed more rapidly to fine-textured, highly organic pond sediments than to sandy riverine sediments, suggesting that organic carbon may be a useful indicator of the potential for selenium to accumulate in sediments and fuel the detrital pathway for food-chain bioaccumulation. Sulfate concentrations did not affect the distribution and bioaccumulation of the selenium mixtures over the range of 80-180 mg/l total sulfate. When each selenium form was radiolabeled and tracked separately, selenomethionine was lost from the water column more rapidly than the inorganic forms. Selenium lost from the water column accumulated primarily in sediments but volatilization was also an important pathway for loss of selenomethionine. Daphnids (crustaceans) accumulated the highest concentrations of selenium followed by periphyton and macrophytes. Selenomethionine was bioaccumulated preferentially over selenate and selenite, suggesting that organoselenium compounds may contribute disproportionately to selenium bioaccumulation and toxicity in aquatic organisms.

The microcosm studies identified selenium speciation processes that were previously unknown or very poorly described and documented. In systems containing only algae, it was determined that over a period of 28 days the water column became permeated with organic selenium even though the original dosing had been with selenate or selenite. Biological processing of the initial inorganic selenium dose produced large amounts of organoselenium that contained an amino-fraction. Due to funding limitations it was not possible to thoroughly fractionate the amino-selenium compounds and determine if free selenomethionine was present. However, the identification of a biological pathway for the production of dissolved amino-selenium is very important and the implications of this finding are clear. Certain environmental conditions may be conducive to the production of organic selenium, perhaps containing free selenomethionine. The potential hazard of selenium will likely be greater in these locations because of enhanced bioaccumulation and toxicity of organic forms of selenium.

Food-chain bioaccumulation studies indicated that all organisms and trophic levels concentrated selenium more strongly from selenomethionine than from selenate or selenite. For the inorganic species, bioconcentration was greatest in algae and successively less in daphnids and bluegill, respectively. However, in waterborne exposures, selenomethionine residues were exceptionally high in daphnids as well as algae, with bioconcentration factors (BCF's) exceeding 350,000 for daphnids and 50,000 for algae. These BCF's were achieved with very low (< 1.0 ug/l) aqueous concentrations of selenomethionine. Resulting tissue residues for these food-chain organisms were in the 5-15 ug/g dry weight range, a concentration that was shown to be toxic in the diet of chinook salmon and bluegill in other drainwater studies summarized in this report. In contrast, the waterborne concentrations of selenate and selenite that were necessary to produce equivalent residue levels in food organisms were in the mg/l range (10 ug/l selenite; 100 mg/l selenate).

Speciation is the key determinant of maximum acceptable levels of selenium in the environment. Thresholds for dietary toxicity and reproductive effects in fish could be attained before waterborne levels of selenium reach 2 ug/l if bioaccumulative organic forms are present. Ten times this amount might be necessary to produce similar problems if only selenate was present. However, the interpretation of dissolved selenium concentrations is not this simple. It is very important to consider that biological processing can convert inorganic selenium to organic forms. Thus, what constitutes an acceptable maximum "total" selenium concentration will depend on the ratio of the chemical forms, their collective (additive) bioaccumulation and toxicity, and their temporal and spatial variability.

The importance of organic selenium in food-chain bioaccumulation is quite clear. Because of the markedly different BCF's for the various selenium forms in lower trophic levels (daphnids and algae), the amount of food-borne selenium potentially available to bluegill or other fish species is much greater if organic selenium is present. The key step in the bioaccumulation process appears to be the amount and chemical form of dissolved selenium available for accumulation by lower trophic levels; this is the point at which BCF's are very high. For fish, dietary selenium is the primary factor in bioaccumulation. Even though BCF's for fish in relation to their diet are generally quite low (0.5-5), the absolute concentration of selenium in the diet can be toxic due to the high BCF's that occur in the lower trophic levels. Low concentrations (<1.0 ug/l) of selenomethionine or other organoselenium compounds likely contribute disproportionately to selenium bioaccumulation and toxicity.

Additional investigations are warranted in regard to several aspects of these studies, including:

1) Documentation of the occurrence and bioavailability of organoselenium compounds -- the studies completed to date found that selenomethionine is bioaccumulated more readily than inorganic selenium. A biological pathway was also identified in which dissolved selenomethionine could be produced. Field studies have found that organoselenium

compounds are present in water and that the concentrations vary both spatially and seasonally. However, the specific compounds present, the mechanisms for their formation, and their bioavailability and toxicity to aquatic biota have not been adequately evaluated. Through the drainwater studies summarized in this report, methodologies have been developed to separate and quantify fractions of dissolved organoselenium compounds, especially the amino-selenium, in water from experimental microcosms. This research, if completed and extended to the field, could describe the formation and release of dissolved organic selenium, including selenomethionine. This information is critical to the completion of a toxicological assessment and hazard evaluation for selenium.

2) Expansion of the selenium bioaccumulation database -- the short duration and limited dose range of selenium used in the bluegill bioaccumulation studies, combined with the limitations of sampling schedules in the toxicity tests, have resulted in some uncertanties regarding selenium uptake kinetics under certain exposure conditions. Although these problems do not affect the clear trends in bluegill selenium bioaccumulation among selenium species and exposure routes, they do reduce the predictive ability of the bioaccumulation models developed in the study. Additional bioaccumulation tests would allow refinement of the models and provide for a more authoritative description of selenium residue dynamics in fish.

Implications for Water Quality Criteria and Resource Management

The current EPA national selenium criterion for the protection of freshwater aquatic life is 5 ug/l as a four-day average, measured on the basis of total selenium. Laboratory data from the drainwater studies reported here indicates that this criterion is not adequate to protect invertebrates and fish if the chemical species of selenium include organic forms such as selenomethionine. There is field data from other studies which indicate that, because of the differential bioaccumulation and effects of waterborne and dietary selenium, a criterion of 5 ug/l may not be adequate to protect sensitive species such as bluegill from reproductive failure. Field studies also support the need for a more stringent criterion on a case-by-case basis. For example, there are clear instances in which the waterborne levels of selenium are well below the 5 ug/l criterion and yet food-chain organisms and predatory fishes and birds have high tissue residues. Studies conducted by the U.S. Department of the Interior at Federal irrigation projects and private drainwater evaporation ponds have documented such discrepancies at several locations in the West. The cause(s) are presently unknown but may be related to the occurrence of organic selenium in low concentrations during part of the year, or perhaps to unique contaminant/water quality interactions that have not been identified.

The laboratory studies reported here indicate that it is possible for biological conversion of waterborne selenium from inorganic to organic forms. If even a small trace of this material is selenomethionine, the potential environmental hazard is clear since less than 1 ug/l can be acutely toxic to fish and can also bioaccumulate over 350,000 times in food organisms and become a dietary toxicant. The unknown factor at this point is whether or not the biological conversion includes the generation of free selenomethionine or other organic forms that produce similar bioaccumulation factors and toxicities.

The 5 ug/l EPA criterion must be applied with caution and should be considered as a starting point for site-specific determinations and development of a final water quality objective or standard. Temporal measurements of the tissue residues of key species need to be coupled with waterborne concentrations to identify locations of special concern that may be influenced by low levels of organic selenium. If discrepancies are found -- i.e., the water levels are only 0-2 ug/l but residues in biota are at or near toxicity thresholds -- additional studies should be conducted to identify the specific sources and pathways for selenium movement and document impacts that may be occurring. Such studies should include the identification of organic selenium compounds, toxicity testing with appropriate invertebrates and fish, and an evaluation of potential bioaccumulation (rates and BCF's) using field and laboratory tests. Aquatic habitats which have a history of selenium inputs coupled with fine, organic-rich sediments and periodic (seasonal) algal blooms may be at high risk for the formation of toxic organic selenium and should be evaluated carefully.

Since 1985, a general guideline has been proposed in the literature which states that the safe level for waterborne selenium, considering food-chain bioaccumulation, lies within the range of 2-5 ug/l. The lower number in this range (2 ug/l) has been adopted by the U.S. Fish and Wildlife Service as the cutoff point for accepting and designating water supplies for wetland management in many areas of the West. For use as a broad Federal-state criterion this guideline is still quite valid. However, with further elucidation of the toxicity and bioconcentration of organic selenium in laboratory studies, coupled with the field data on food-chain bioaccumulation, it is evident that there are some exceptions. The development and enforcement of state and local water quality standards requires close scrutiny of factors that may justify relaxing or tightening the national criterion. It is essential that aquatic resource managers and environmental regulators review monitoring and evaluation programs carefully in order to identify cases in which site-specific assessments and standards are warranted.

INTRODUCTION

In March 1986 the U.S. Fish and Wildlife Service (USFWS) and the U.S. Bureau of Reclamation entered into an intra-agency agreement (6-AA-20-04170) for the purpose of supporting research on the effects of agricultural drainwater contaminants on fish and wildlife populations. The agreement initiated a multi-year research effort to be conducted by USFWS research centers in support of the San Joaquin Valley Drainage Program.

The National Fisheries Contaminant Research Center (NFCRC, headquartered in Columbia, MO) received responsibility for seventeen major studies included in this agreement. Central to this research thrust was the determination of contaminant movement and localization in aquatic ecosystems, particularly the phenomenon of bioaccumulation and resultant exposure of fish and wildlife through the food chain. Study design included both field and laboratory methodologies in order to isolate and identify specific chemical, physical, and biological factors influencing contaminant behavior and toxicity. All of the studies were interrelated to provide a comprehensive approach to elucidating the impacts of drainwater contaminants on fish and wildlife populations. This report summarizes the findings of the NFCRC studies.

SYNOPTIC SURVEY OF CONTAMINANTS IN SAN JOAQUIN RIVER FISHES

Introduction

About 100,000 ha of irrigated croplands in the San Joaquin Valley are now affected either by inadequate drainage of salts and leaching water or because the drainage water being disposed is adversely affecting Valley lands, the San Joaquin River, and/or its tributaries. Between 1978 and 1986, drainage from 3,200 ha west of Mendota was discharged into Kesterson Reservoir by way of the San Luis Drain. The discovery that tile drainage entering the San Luis Drain contained elevated concentrations of selenium led to the suspension of further releases of drainage water into the Drain and the closure of Kesterson Reservoir in June 1986. Historically, irrigation water supplemented by a mixture of surface return flows and tile drainage from about 31,000 ha north of Mendota was delivered to the Grassland Water District for use in flooding marsh ponds on federal and state wildlife refuges and privately owned duck clubs. Beginning in the fall of 1985, however, many waterfowl managers ceased using drainage water to flood their lands after learning of the high levels of selenium and other trace elements in tile drainage. Currently, nearly all drainage water entering the Grasslands are collected into two major sloughs (Mud Slough (north) and Salt Slough) that feed directly into the San Joaquin River. The San Joaquin River, in turn, discharges into the Sacramento-San Joaquin Delta and San Francisco Bay.

Farmlands totaling at least 67,000 ha need drainage and the farmers are searching for other means or places to dispose of their drainage water. Some of this drainage is currently stored on individual farms in small, unlined evaporation ponds. The land needing drainage is expected to increase to 500,000 ha by the year 2000, or about 40% of the land within the Federal (Central Valley Project, CVP) irrigation service area (San Joaquin Valley Drainage Program, 1990).

Earlier discoveries of high levels of selenium in water (up to 330 μg/L) and biota (up to 370 μg/g dry weight) from the San Luis Drain and Kesterson Reservoir have raised concerns about the present methods of drainage disposal. This trace element occurs naturally in the soil and is believed to cause the high mortalities and deformities observed in waterfowl at Kesterson Reservoir and elsewhere in the San Joaquin Valley. Effects on fish and other wildlife are still poorly understood; however, studies conducted in selenium-polluted environments elsewhere in the United States suggest that residue levels ≥ 12 μg/g in the whole bodies of sensitive freshwater fishes may be sufficient to cause reproductive failure and other toxic effects (Lemly and Smith, 1987).

Recent studies in the San Joaquin Valley indicate that fish populations outside of the San Luis Drain and Kesterson Reservoir are contaminated with high levels of selenium and other inorganic and organic chemicals (Saiki and May, 1988). However, the geographic (spatial) patterns and severity of contamination are still poorly defined. Contaminant concentrations must be determined not only to identify "hot spots" and establish current baseline conditions for subsequent surveys, but also to provide reference points for field and laboratory studies that examine cause-and-effect relations between contaminant burdens and biological effects.

The primary objective of this investigation was to determine if elements accumulated by several species of fish from the San Joaquin Valley were elevated in comparison to "background" concentrations established by previous surveys in other watersheds. Secondary objectives were to determine if the elemental concentrations in fish were related to inflows of tile drainwater into the San Joaquin River, and if the concentrations had increased over the past 5 yr due to recent changes in the management and use of tile drainwater in the Grasslands. Because of potential public health concerns, we were also interested in determining if elemental concentrations in fish varied according to tissue type (fillets and whole body).

Study Area

Twenty-seven collection sites were established on selected reaches of the San Joaquin River and its tributaries, with an additional reference site located in the Sacramento River system at Knights Landing (Figure 1). All of the sites in the San Joaquin River and west-side tributaries are affected to differing degrees by tile drainage water. Flows in the Sacramento and San Joaquin rivers and the eastern tributaries of the San Joaquin River originate in the Sierra Nevada; these rivers receive their initial water supplies mainly from snowmelt and rainfall. During the irrigation season (usually March to October), irrigation water from the Delta-Mendota Canal and return flows from irrigated fields usually contribute most of the discharge in downstream portions of the San Joaquin River. In contrast, west-side tributaries of the San Joaquin River--some of which originate on the San Joaquin Valley floor--derive most of their discharge from groundwater seepage and irrigation return flows (Gilliom, 1986; Presser and Barnes, 1985).

Methods

We attempted to collect five fish or a total of at least 50 g for each composite sample. All fish were captured in September-December 1986 with seines, gill nets, electrofishing gear, or a combination of these methods. Immediately after capture, the fish were rinsed with water from the respective sites, wrapped and bagged in plastic, then chilled on ice. At the field station, the fish were weighed and measured (total length), then rewrapped and bagged in plastic and frozen (-10°C). Samples remained frozen until they were prepared for chemical analyses.

A total of 285 composite samples of either whole fish, carcasses, fillets, or gonads were analyzed for moisture and trace elements at the National Fisheries Contaminant Research Center in Columbia, Missouri. Large fish ($\geq$150 g wet tissue) were band-sawed into smaller chunks, the chunks were homogenized with a meat grinder, then about 150 g were lyophilized; smaller fish (< 150 g) were first opened with bone shears, then the entire fish was lyophilized. Moisture content of each sample was determined during the lyophilization process. The dried samples were homogenized by grinding into a fine powder with either a Moulenix Le Petite grinder or an Osterizer blender.

Chemical preparation and analysis techniques on the dried samples depended upon the element being determined. Digestates were analyzed for boron by inductively coupled argon plasma emission spectrometry, whereas molybdenum was analyzed by graphite furnace atomic absorption spectrophotometry. Samples for arsenic and selenium determinations were prepared by dry-ashing, then the analyses were performed by hydride generation atomic absorption spectrophotometry. Samples for analysis of chromium and mercury were prepared with a nitric acid-heated water bath technique, then the digestates were analyzed for chromium by Zeeman graphite furnace atomic absorption spectrophotometry and for mercury by cold vapor atomic absorption spectrophotometry. All digestate concentration values provided by the Perkin-Elmer and Varian instrumentation were entered into a Perkin-Elmer 3600 data station or a VAX 20/20 terminal for computation of the final concentration results.

Principal Findings

In general, the moisture content of whole-fish samples differed significantly among both species ($P \leq 0.05$ in 14 of 24 one-way ANOVA comparisons) and sites ($P \leq 0.05$ in 2 of 4 one-way ANOVA comparisons). Moisture content in samples of bluegills, common carp, and largemouth bass was inversely correlated with total length and weight of the fish ($P \leq 0.01$ in all instances); similar relations were not evaluated for mosquitofish because lengths and weights of individuals from this species were not measured, and for Sacramento blackfish because only three samples were analyzed. To standardize the moisture content of all samples, we report the concentrations of trace elements on a dry weight basis, unless indicated otherwise.

General comparisons: Detectable concentrations of all trace elements--arsenic, boron, chromium, mercury, molybdenum, and selenium--were measured in one or more samples of whole bluegills, common carp, mosquitofish, largemouth bass, and Sacramento blackfish (Tables 1-6). Based on samples from all sites combined, the geometric mean concentrations (µg/g, ranges in parentheses) for each element follow. Bluegills: arsenic, 0.423 (0.20-1.4); boron, 1.18 (0.81-5.6); chromium, 0.369 (0.029-2.1); mercury, 0.279 (0.11-0.52); molybdenum, 0.293 (0.13-0.92); and selenium, 1.92 (0.38-9.4). Carp: arsenic, 0.337 (0.13-1.6); boron, 1.10 (0.81-2.8); chromium, 0.447 (0.034-1.5); mercury, 0.279 (0.056-0.80); molybdenum, 0.451 (0.12-1.0); and selenium, 2.25 (0.50-10.). Mosquitofish: arsenic, 0.756 (0.29-2.1); boron, 1.40 (0.81-7.6); chromium, 0.452 (0.053-3.8); mercury, 0.233 (0.12-0.50); molybdenum, 0.249 (0.13-0.70); and selenium, 1.81 (0.45-11.). Largemouth bass: arsenic, 0.260 (0.14-0.61); boron, 0.912 (0.81-1.5); chromium, 0.243 (0.034-4.3); mercury, 0.644 (0.21-1.9); molybdenum, 0.280 (0.13-0.69); and selenium, 1.53 (0.88-9.7). Sacramento blackfish: arsenic, 0.678 (0.66-0.70); boron, no data; chromium, 2.28 (1.8-3.0); mercury, 0.0593 (0.052-

0.068); molybdenum, no data; and selenium, 3.87 (3.7-4.1).

The levels of arsenic, mercury, and selenium are routinely monitored in whole freshwater fish from throughout the United States by the National Contaminant Biomonitoring Program (NCBP). The 85th percentile concentrations from the NCBP represent arbitrary values that are useful for identifying locations where concentrations of elements in fish are unusually high, but do not necessarily imply an associated toxic effect. For two elements where toxicological effects have been related to concentrations in whole fish--e.g., cadmium (Eisler 1985) and selenium (Gillespie and Baumann 1986; Lemly and Smith 1987)--the toxic concentrations are much higher than the NCBP 85th percentile concentrations.

The 1976-84 NCBP 85th percentile values for arsenic ranged from 0.22 to 0.38 μg/g wet weight (about 0.85-1.5 μg/g dry weight, assuming 74% moisture; Schmitt and Brumbaugh, in press). Moore and Ramamoorthy (1984) also reported that fish from unpolluted or mildly contaminated waters generally contain <0.4 μg/g wet weight (about 1.5 μg/g dry weight) of arsenic. Compared to concentrations measured in fishes from our survey, only mosquitofish from one site in the Grassland Water District (i.e., Salt Slough at the San Luis National Wildlife Refuge) contained elevated arsenic levels (Table 1). Potential sources of arsenic to fish in the San Joaquin River system include arsenical herbicides and defoliants used in agriculture.

The 1976-84 NCBP 85th percentile concentrations for mercury ranged from 0.17 to 0.19 μg/g wet weight (about 0.65-0.73 μg/g dry weight; Schmitt and Brumbaugh, in press). Mercury concentrations in bluegills, common carp, and mosquitofish from all sites that we sampled were generally at or below the NCBP 85th percentile concentrations (Table 4); in contrast, largemouth bass from 2 of 3 sites in the San Joaquin River (at Hills Ferry and at

Durham Ferry) and from the Tuolumne and Stanislaus rivers exceeded the NCBP 85th percentile concentrations. However, none of the concentrations exceeded 1.1 μg/g fresh weight (about 4.2 μg/g dry weight) which, according to Eisler (1987), represents evidence of an environmental mercury problem.

The 1976-84 NCBP 85th percentile values for selenium ranged from 0.70-0.82 μg/g wet weight (about 2.7-3.2 μg/g dry weight; Schmitt and Brumbaugh, in press). Bluegills, common carp, mosquitofish, largemouth bass, and Sacramento blackfish collected from up to 5 sites in the Grassland Water District (i.e., Mud Slough at the Los Banos Wildlife Area, Mud Slough at Gun Club Road, Camp 13 Ditch, Agatha Canal, and the Helm Canal) and 1 site in the San Joaquin River (at Fremont Ford) had selenium concentrations that exceeded the NCBP 85th percentile values (Table 6). According to Lemly and Smith (1987), biological symptoms of selenium contamination (e.g., reproductive failure--whether through effects on the adult ovary or on the embryonic development and survival of young) may first occur in sensitive freshwater fishes such as centrarchids at whole-body concentrations $\geq$12 μg/g. Concentrations found in this study sometimes approached (9-11 ug/g, Table 6), but did not exceed this level.

Boron, chromium, and molybdenum have not been measured by the NCBP or other nationwide contaminant surveys. However, based on data from a limited number of field surveys, Saiki and May (1988) suggested that whole freshwater fish typically contain <4 μg/g boron. During our survey, only mosquitofish from Mud Slough at Gun Club Road exceeded 4 μg/g of boron (Table 2).

According to Wiener et al. (1984), chromium levels $\geq$3.5 μg/g in whole bluegills possibly represent elevated concentrations, whereas Eisler (1986) indicated that tissue levels >4.0 μg/g should be viewed as presumptive evidence of chromium contamination.

All of the fishes analyzed during our survey contained mean chromium concentrations ≤ 3.0 µg/g (Table 3), suggesting that this element was not excessively elevated.

Although a baseline concentration for molybdenum in whole freshwater fish has not been determined, rainbow trout (Oncorhynchus mykiss, previously identified as Salmo gairdneri) held in laboratory water containing 6 µg/L (freshwater generally contains < 10 µg/L; Friberg et al. 1975, cited by Eisler 1989) accumulated up to 0.146 µg/g wet weight (about 0.56 µg/g dry weight) in their tissues (Ward 1973). The maximum concentration of molybdenum measured during our survey was 1.0 µg/g (Table 5), suggesting that this element might be slightly elevated in samples from some sites. Although excessive concentrations of molybdenum can induce copper and sulfate deficiencies in mammals (especially domestic ruminants), the toxicological effects of this element in fish tissues are unknown (Eisler 1989).

Relation to inflows of tile drainage water: Water quality surveys in the San Joaquin River system have revealed longitudinal (upstream-downstream) patterns for several physicochemical variables in the San Joaquin River that suggest progressively increasing environmental degradation caused by inflows of agricultural drainwater (Saiki and Palawski, in press). In particular, turbidity, total alkalinity, total hardness, total dissolved solids, and conductivity increase at downstream sampling sites that receive the most concentrated flows of agricultural drainage water. In 1985, Salt and Mud (north) sloughs collectively supplied only 12% of the total flow in the San Joaquin River between Lander Avenue and its confluence with the Merced River; however, these sloughs contributed about 81% of the selenium, 69% of the boron, 44% of the molybdenum, and 46% of the dissolved salts occurring in this reach of the river (State Water Resources Control Board 1987).

The concentrations of five elements--arsenic, boron, chromium, mercury, and selenium--differed significantly ($\underline{P} \leq 0.05$) in three or more of the fishes that we sampled from among the various regions (Tables 1-4 and 6). Unlike other elements, the concentrations of molybdenum did not vary significantly among regions (Table 5). Only selenium varied in a recognizable pattern that seemingly corresponded with inputs of tile drainwater into the San Joaquin River and its tributaries.

Selenium: Recognizable geographic patterns were observed in the distribution of selenium. Except for mosquitofish collected from Salt Slough at Hereford Road and the Helm Canal, and bluegills, common carp, and mosquitofish collected from Los Banos Creek, the mean concentrations of selenium in fishes from the Grasslands exceeded 2.7 μg/g and were generally higher than in other regions of the San Joaquin Valley. Common carp and mosquitofish from two sites on the San Joaquin River (i.e., at Fremont Ford and above Hills Ferry Road) that lie immediately downstream from the Grasslands also contained mean selenium concentrations exceeding 2.7 μg/g. According to the State Water Resources Control Board (1987), tile drainage from slightly more than 38,000 ha of croplands located south (upslope) of the Grasslands accounts for most of the selenium contamination in the Grasslands and San Joaquin River. Presser and Barnes (1985) reported that agricultural sumps in the area, which empty into drains that eventually flow into the Grasslands, contain dissolved selenium at concentrations as high as 4,200 μg/L.

With two exceptions, fishes collected from the San Joaquin River above the Grasslands and from upstream tributaries that enter the San Joaquin River at Mendota Pool (e.g., Fresno Slough, Delta-Mendota Canal) typically contained <2.0 μg/g of selenium. The exceptions were bluegills from Highway 152 and common carp from Firebaugh which averaged 2.06 μg/g and 2.50 μg/g of selenium, respectively. Although the San Joaquin River immediately upstream from the Grasslands (at Lander Avenue) receives low exposure (<1 μg/L) to dissolved selenium (Gilliom 1986), previous surveys of elemental concentrations in resident and anadromous fishes suggest that local enrichment of selenium--perhaps from undocumented discharges of tile drainwater, seepage from municipal sewage treatment ponds, or other sources--probably occurs above Lander Avenue in the reach extending from Firebaugh to Highway 152 (Saiki and May 1988; Saiki and Palawski, in press).

Farther downstream on the San Joaquin River between Crows Landing and Durham Ferry, mean concentrations of selenium generally averaged <1.8 μg/g, or approximately two-thirds of the concentrations measured in the Fremont Ford-Hills Ferry vicinity. Lower selenium concentrations in fish from this reach of the San Joaquin River might result from dilution of tile drainwater by low-selenium waters from the Merced, Tuolumne, and Stanislaus rivers; uptake by organisms not in the fish food chain; and losses due to volatility and sequestering in sediments.

Selenium concentrations in fishes from the remaining tributaries (Merced, Tuolumne, Stanislaus, and Sacramento rivers, and Orestimba Creek) were all low, averaging ≤ 1.8 μg/g. Dissolved concentrations of selenium from these waters are also low, ranging up to 2 μg/L (Gilliom 1986; Shelton and Miller 1988).

Temporal patterns: For more than 30 years, agricultural drainwater (a mixture of irrigation water supplemented by surface return flows and tile drainage) was delivered to the Grasslands for flooding marsh ponds on federal and state wildlife refuges and privately owned duck clubs, then released for disposal into Salt and Mud (north) sloughs. The use of agricultural drainwater for managing wetlands may have removed more than one-half of the total selenium load that otherwise would have entered the San Joaquin River (State Water Resources Control Board 1987).

Beginning in the fall of 1985, many waterfowl managers ceased using agricultural drainwater after learning that it contained high concentrations of selenium and other trace elements (e.g., boron, cadmium, chromium, and zinc). Since that time, agricultural drainwater has been shunted directly into Salt and Mud sloughs.

The concentrations of arsenic, mercury, and selenium have been measured annually since 1984 in fish from the Grasslands by the National Fisheries Contaminant Research Center. In addition, fish from one location (Mud Slough at the Los Banos Wildlife Area) have been collected periodically since 1969 for determinations of these elements as part of the National Contaminant Biomonitoring Program (previously referred to as the National Pesticide Monitoring Program). As judged by the data, only selenium concentrations have varied with time in the Grasslands. With few exceptions, the mean concentrations of this element seemingly peaked in 1984, with lower concentrations measured in fish since that time. This pattern is unusual because average concentrations of dissolved selenium in water flowing through the Grasslands increased from about 17 μg/L in water-year 1984 (October 1983 to September 1984) to 22 μg/L in water-year 1985 (October 1984 to September 1985; estimated from monthly projections of selenium concentrations and discharge rates given by the State Water Resources Control Board 1987). More recently (April 1986 to March 1987), dissolved selenium has averaged about 9.7 μg/L (range, <1-23 μg/L) in Salt Slough at Lander Avenue, and about 13.3 μg/L (range, <1-31 μg/L) in Mud Slough (north) downstream from Gun Club Road (Shelton and Miller 1988).

The apparent discrepancy between the observed and expected temporal patterns of selenium in fish is still unresolved. Perhaps the analytical procedures used to measure selenium concentrations in fish somehow yielded erroneous results. This possibility is highly unlikely because the quality-assurance and quality-control data were within acceptable limits. Another possibility is that the limited numbers of fish samples (generally 1-4 composite samples for each species and site that did not consider sex and age differences) analyzed during each year were inadequate for detecting small changes in selenium concentrations.

Relation of elemental concentrations in fillets and whole fish: In order to assist public health agencies with assessments of human-health risks in people who consume fish from waters in the San Joaquin Valley, we estimated the relations between arsenic, mercury, and selenium concentrations in whole fish with those in their fillets (Table 7). In general, the concentrations of these elements were lower in whole-body samples than in their corresponding fillets. We did not estimate the relations for boron, chromium, and molybdenum because they were not significantly correlated ($P > 0.05$).

Conclusions and Research Needs

High concentrations of trace elements can adversely affect the reproduction, growth, or survival of fish, and lead to public health advisories for humans who eat affected fish. Our survey demonstrated that fish from certain portions of the Grasslands and the San Joaquin River adjacent to or immediately downstream from the Grasslands have accumulated excessive concentrations of selenium. The available data also indicated that the selenium originated from agricultural drainwater flowing through the Grasslands.

To better understand and predict the consequences of discharging seleniferous agricultural drainwater into the San Joaquin River or its tributaries, scientists need additional information about several aspects of selenium bioaccumulation and its effects on fish. Five examples follow:

1. The chemical forms of selenium--selenate, selenite, elemental selenium, and

selenide (including organic selenium)--that occur in water and forage organisms should be determined so that appropriate form(s) can be used when fish are exposed to this element in laboratory toxicity experiments.

2. Better knowledge is needed of selenium concentrations that occur in different components of the aquatic food chain as a result of exposure to various concentrations of waterborne selenium. Also, the biochemical and physiological processes that underlie the uptake and excretion of selenium at each trophic level in the food chain must be understood to enable the development of predictive models of selenium bioaccumulation.

3. Threshold waterborne and dietary concentrations of selenium that affect the survival, growth, reproduction, and other biological processes of fish must be determined. Effects should be studied throughout the complete life cycle of several ecologically and economically important fish species, and preferably through several generations, because not all adverse effects might be expressed over a shorter time period.

4. Although field and laboratory studies have documented the occurrence of mortality and teratogenic effects in fish exposed to high-selenium environments, there remains a need for better diagnostic indicators of selenium toxicity. These should include sublethal characteristics (e.g., behavior) that can be measured in live animals and also those that are diagnostic of selenium-induced mortality.

5. Knowledge of how selenium interacts with other contaminants must be gained if the ecological significance of selenium is to be accurately assessed. It is known that the toxic effects of selenium can be greatly altered by other chemicals (such as heavy metals and other trace elements), or factors such as water quality variables, species of organism and its life stage, nutritional status, disease state, and level of stress.

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Table 1. Arsenic concentrations (µg/g dry weight) in five fishes from the Sacramento and San Joaquin valleys, September-December, 1986. N, sample size (no. detected/no. analyzed); $\bar{X}$, mean (geometric mean if N >1); R, range. Refer to Figure 1 for locations of collection sites[a].

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR1												
1 SJRFW	3/3	0.263	0.22-0.36	3/3	0.382	0.35-0.43	3/3	0.553	0.54-0.57	0/0	--	--
2 SJR145	3/3	0.313	0.20-0.41	1/1	0.46	--	3/3	0.812	0.79-0.82	0/0	--	
	$\bar{X}$ = 0.287 C			$\bar{X}$ = 0.400 A			$\bar{X}$ = 0.670 AB			$\bar{X}$ =	--	
SJR2												
3 SJRMP	3/3	0.321	0.30-0.36	0/0	--	--	3/3	0.296	0.29-0.32	0/0	--	--
4 SJRFB	3/3	0.315	0.22-0.39	3/3	0.368	0.32-0.47	3/3	0.838	0.82-0.86	0/0	--	--
5 SJR152	3/3	0.603	0.55-0.70	0/0	--	--	3/3	0.836	0.81-0.87	0/0	--	--
6 SJR165	5/5	0.351	0.28-0.45	2/2	0.168	0.13-0.22	3/3	0.572	0.55-0.61	3/3	0.322	0.29-0.38
	$\bar{X}$ = 0.378 BC			$\bar{X}$ = 0.269 A			$\bar{X}$ = 0.587 B			$\bar{X}$ = 0.322 AB		
SJR3												
7 SJRFF	3/3	0.541	0.43-0.76	3/3	0.421	0.39-0.49	3/3	1.15	1.1 -1.2	0/0	--	--
8 SJRHF	7/7	0.416	0.28-0.87	3/3	0.437	0.35-0.53	3/3	0.764	0.71-0.83	3/3	0.208	0.16-0.27
	$\bar{X}$ = 0.450 B			$\bar{X}$ = 0.429 A			$\bar{X}$ = 0.938 AB			$\bar{X}$ = 0.208 B		
SJR4												
9 SJRCL	3/3	1.12	0.89-1.4	3/3	0.585	0.55-0.62	3/3	0.910	0.87-0.94	0/0	--	--
10 SJRLP	3/3	1.27	1.3 -1.3	2/2	0.363	0.34-0.39	3/3	1.50	1.5 -1.6	0/0	--	--

Table 1. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR4 (Continued)												
11 SJR132	2/2	0.937	0.91-0.97	2/2	0.790	0.39-1.6	3/3	1.05	1.0 -1.1	0/0	--	--
12 SJRDF	6/6^b	0.900	0.62-1.1	3/3	0.348	0.30-0.46	3/3	1.28	1.2 -1.3	3/3	0.302	0.30-0.31
	$\bar{X}$ = 1.02 A			$\bar{X}$ = 0.483 A			$\bar{X}$ = 1.16 A			$\bar{X}$ = 0.302 AB		
TRIB1												
13 FS	3/3	0.332	0.31-0.37	0/0	--	--	3/3	0.618	0.51-0.87	0/0	--	--
14 DMC	0/0	--	--	0/0	--	--	3/3	0.587	0.57-0.60	0/0	--	--
15 OC	0/0	--	--	1/1	0.28	--	3/3	0.420	0.41-0.44	0/0	--	--
	$\bar{X}$ = 0.332 BC			$\bar{X}$ = 0.28 A			$\bar{X}$ = 0.534 B			$\bar{X}$ = --		
TRIB2												
16 MSLBWA	7/7	0.347	0.26-0.49	3/3	0.209	0.17-0.31	3/3	0.555	0.55-0.56	0/0	--	--
17 SSHR	0/0	--	--	0/0	--	--	3/3	1.20	1.2 -1.3	0/0	--	--
18 SSSLNWR	2/2	0.371	0.23-0.61	3/3	0.410	0.26-0.56	3/3	2.04	1.9 -2.1	3/3	0.250	0.21-0.34
19 C13D	0/0	--	--	0/0	--	--	3/3	0.840	0.79-0.90	0/0	--	--
20 MSGCR	5/5	0.336	0.21-0.48	3/3	0.197	0.14-0.31	3/3	0.718	0.70-0.74	2/2	0.293	0.25-0.35
21 AC	3/3	0.416	0.38-0.47	3/3	0.229	0.16-0.30	3/3	1.09	1.0 -1.2	0/0	--	--
22 HC	1/1	0.35	--	0/0	--	--	3/3	0.447	0.41-0.48	0/0	--	--
23 MC	0/0	--	--	0/0	--	--	3/3	0.635	0.55-0.72	0/0	--	--
24 LBC	3/3	0.318	0.27-0.43	3/3	0.458	0.31-0.72	3/3	1.10	1.1 -1.1	0/0	--	--
	$\bar{X}$ = 0.352 BC			$\bar{X}$ = 0.282 A			$\bar{X}$ = 0.868 AC			$\bar{X}$ = 0.266 AB		

Table 1. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}$	R	N	$\bar{X}$	R	N	$\bar{X}$	R	N	$\bar{X}$	R
TRIB3												
25 MR	3/3	0.279	0.21-0.33	3/3	0.236	0.22-0.25	3/3	0.788	0.53-0.96	3/3	0.185	0.14-0.21
26 TR	3/3	0.255	0.22-0.29	1/1	0.21	--	3/3	0.422	0.41-0.44	3/3	0.213	0.19-0.23
27 SR	3/3	0.271	0.20-0.34	0/0	--	--	3/3	0.602	0.57-0.63	3/3	0.227	0.21-0.27
	$\bar{X}$ = 0.268 C			$\bar{X}$ = 0.229 A			$\bar{X}$ = 0.585 BC			$\bar{X}$ = 0.208 B		
SR												
28 SAC	3/3	0.390	0.34-0.49	3/3	0.315	0.27-0.40	3/3	0.535	0.52-0.56	3/3	0.428	0.29-0.61
	$\bar{X}$ = 0.390 BC			$\bar{X}$ = 0.315 A			$\bar{X}$ = 0.535 BC			$\bar{X}$ = 0.428 A		
$f(df_1,df_2)^d$	27.61**			2.63*			6.66**			6.85**		

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[a] Arsenic values for Sacramento blackfish at MSLBWA are N = 3/3, $\bar{X}$ = 0.678, and R = 0.66-0.70.

[b] Within a column, geometric grand means with the same capital letter are not significantly different (P >0.05, Tukey-Kramer hsd test).

[c] One bluegill composite sample was not included due to the absence of data on the concentration of arsenic in gonads.

[d] For bluegills, df1 = 7, df2 = 72; for common carp, df1 = 7, df2 = 40; for mosquitofish, df1 = 7, df2 = 76; for largemouth bass, df1 = 5, df2 = 20; * $P \leq 0.05$, ** $P \leq 0.01$.

Table 2. Boron concentrations (µg/g dry weight) in five fishes from the Sacramento and San Joaquin valleys, September-December, 1986. N, sample size (no. detected/no. analyzed); $\bar{X}$, mean (geometric mean if N >1); R, range. Refer to Figure 1 for locations of collection sites[a].

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR1												
2 SJR145	0/3	<3.06	<3.1-<3.1	0/1	<3.1	--	0/3	<3.06	<3.1-<3.1	0/0	--	--
	$\bar{X}$ = <3.06 AB			$\bar{X}$ = <3.1 A			$\bar{X}$ = <3.06 B			$\bar{X}$ = --		
SJR2												
6 SJR165	0/3	<1.62	<1.6-<1.6	0/2	<1.62	<1.6-<1.6	0/3	<1.62	<1.6-<1.6	1/3	0.890	<1.6-1.1
	$\bar{X}$ = <1.62 B			$\bar{X}$ = <1.62 A			$\bar{X}$ = <1.62 D			$\bar{X}$ = 0.890 B		
SJR3												
7 SJRFF	0/3	<3.06	<3.1-<3.1	0/3	<3.06	<3.1-<3.1	0/3	<3.06	<3.1-<3.1	0/0	--	--
8 SJRHF	0/3	<1.65	<1.7-<1.7	0/3	<1.65	<1.7-<1.7	1/3	1.06	<1.7-1.8	0/3	<1.65	<1.7-<1.7
	$\bar{X}$ = <2.25 B			$\bar{X}$ = <2.25 A			$\bar{X}$ = 1.27 BC			$\bar{X}$ = <1.65 B		
SJR4												
9 SJRCL	0/0	--	--	0/0	--	--	0/3	<3.06	<3.1-<3.1	0/0	--	--
	$\bar{X}$ = --			$\bar{X}$ = --			$\bar{X}$ = <3.06 B			$\bar{X}$ = --		
TRIB2												
20 MSGCR	2/3	2.98	<3.1-5.6	2/3	1.72	<1.7-2.8	3/3	7.03	6.1-7.6	0/2	<3.06	<3.1-<3.1
	$\bar{X}$ = 2.98 A			$\bar{X}$ = 1.72 A			$\bar{X}$ = 7.03 A			$\bar{X}$ = <3.06 A		

Table 2. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
TRIB3												
26 TR	0/3	≤1.62	<1.6-<1.6	0/1	≤1.6	--	0/3	≤1.62	<1.6-<1.6	0/3	≤1.62	<1.6-<1.6
	$\bar{X}$ = <1.62 B			$\bar{X}$ = <1.6 A			$\bar{X}$ = 1.62 D			$\bar{X}$ = <1.62 B		
SR												
28 SAC	0/3	≤1.65	<1.7-<1.7	0/3	≤1.65	<1.7-<1.7	0/3	≤1.65	<1.7-<1.7	0/3	≤1.65	<1.7-<1.7
	$\bar{X}$ = <1.65 B			$\bar{X}$ = <1.65 A			$\bar{X}$ = <1.65 CD			$\bar{X}$ = <1.65 B		
$f(df_1,df_2)^c$		8.54**			1.77			49.06**			27.19**	

[a] Boron values for Sacramento blackfish at MSLBWA are N = 0/0, $\bar{X}$ = --, and R = --.

[b] Within a column, geometric grand means with the same capital letter are not significantly different (P >0.05, Tukey-Kramer hsd test).

[c] For bluegills, df1 = 5, df2 = 15; for common carp, df1 = 5, df2 = 10; for mosquitofish, df1 = 6, df2 = 17; for largemouth bass, df1 = 4, df2 = 9; **P ≤0.01.

Table 3. Chromium concentrations (µg/g dry weight) in five fishes from the Sacramento and San Joaquin valleys, September-December, 1986. N, sample size (no. detected/no. analyzed); $\bar{X}$, mean (geometric mean if N >1); R, range. Refer to Figure 1 for locations of collection sites[a].

Region, collection site,		Bluegills			Common carp			Mosquitofish			Largemouth bass	
and ANOVA	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR1												
1 SJRFW	1/3	0.0850	<0.13-0.14	0/3	<0.131	<0.13-<0.13	0/3	<0.131	<0.13-<0.13	0/0	--	--
2 SJR145	0/3	<0.150	<0.15-<0.15	1/1	0.58	--	3/3	0.878	0.73-1.1	0/0	--	--
	$\bar{X}$ = 0.080 C			$\bar{X}$ = 0.113 B			$\bar{X}$ = 0.240 A			$\bar{X}$ = --		
SJR2												
3 SJRMP	3/3	0.391	0.35-0.46	0/0	--	--	3/3	0.199	0.10-0.46	0/0	--	--
4 SJRFB	2/3	0.131	<0.057-0.34	3/3	0.453	0.36-0.57	3/3	0.665	0.56-0.73	0/0	--	--
5 SJR152	3/3	0.471	0.35-0.68	0/0	--	--	3/3	0.107	0.086-0.13	0/0	--	--
6 SJR165	3/3	0.786	0.53-0.98	1/2	0.147	<0.068-0.64	3/3	0.537	0.44-0.66	2/3	0.215	<0.07-0.58
	$\bar{X}$ = 0.371 AB			$\bar{X}$ = 0.289 AB			$\bar{X}$ = 0.296 A			$\bar{X}$ = 0.215 A		
SJR3												
7 SJRFF	3/3	0.699	0.56-0.89	3/3	0.764	0.65-0.97	3/3	0.919	0.81-1.2	0/0	--	--
8 SJRHF	3/3	0.523	0.44-0.66	2/3	0.402	<0.24-1.4	3/3	0.758	0.60-1.1	2/3	0.269	<0.24-0.43
	$\bar{X}$ = 0.604 AB			$\bar{X}$ = 0.554 AB			$\bar{X}$ = 0.835 A			$\bar{X}$ = 0.269 A		
SJR4												
9 SJRCL	3/3	0.890	0.53-1.3	3/3	0.978	0.76-1.4	3/3	0.898	0.76-1.0	0/0	--	--
10 SJRLP	3/3	1.38	0.79-2.1	2/2	0.815	0.72-0.93	3/3	2.83	2.6 -3.0	0/0	--	--

Table 3. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR4 (Continued)												
11 SJR132	2/2	0.524	0.38-0.73	2/2	0.566	0.41-0.78	3/3	0.393	0.39-0.41	0/0	--	--
12 SJRDF	3/3	0.228	0.18-0.33	3/3	0.570	0.44-0.80	3/3	0.317	0.26-0.38	3/3	0.197	0.058-0.48
	$\bar{X}$ = 0.628 A			$\bar{X}$ = 0.719 A			$\bar{X}$ = 0.750 A			$\bar{X}$ = 0.197 A		
TRIB1												
13 FS	3/3	0.749	0.68-0.88	0/0	--	--	3/3	0.213	0.14-0.35	0/0	--	--
14 DMC	0/0	--	--	0/0	--	--	3/3	0.250	0.19-0.31	0/0	--	--
15 OC	0/0	--	--	1/1	0.66	--	3/3	0.364	0.19-0.83	0/0	--	--
	$\bar{X}$ = 0.749 AB			$\bar{X}$ = 0.66 AB			$\bar{X}$ = 0.269 A			$\bar{X}$ = --		
TRIB2												
16 MSLBWA	3/3	0.354	0.29-0.44	2/3	0.142	<0.13-0.22	0/3	<0.130	<0.13-<0.13	0/0	--	--
17 SSHR	0/0	--	--	0/0	--	--	3/3	0.993	0.87-1.2	0/0	--	--
18 SSSLNWR	2/2	0.312	0.27-0.36	3/3	0.602	0.47-0.69	3/3	0.801	0.76-0.88	3/3	0.199	0.06-0.39
19 C13D	0/0	--	--	0/0	--	--	3/3	0.572	0.44-0.66	0/0	--	--
20 MSGCR	3/3	0.488	0.29-0.65	3/3	0.862	0.30-1.5	3/3	1.02	0.85-1.3	0/2	<0.242	<0.24-<0.24
21 AC	3/3	0.392	0.25-0.67	2/3	0.251	<0.13-0.50	3/3	3.04	2.2 -3.8	0/0	--	--
22 HC	1/1	0.34	--	0/0	--	--	3/3	0.307	0.26-0.44	0/0	--	--
23 MC	0/0	--	--	0/0	--	--	3/3	1.07	0.75-1.5	0/0	--	--
24 LBC	3/3	0.439	0.24-0.78	3/3	0.883	0.76-1.0	3/3	0.394	0.35-0.43	0/0	--	--
	$\bar{X}$ = 0.395 AB			$\bar{X}$ = 0.439 AB			$\bar{X}$ = 0.611 A			$\bar{X}$ = 0.163 A		

Table 3. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
TRIB3												
25 MR	3/3	0.171	0.11-0.29	3/3	0.399	0.16-0.68	3/3	0.271	0.19-0.35	3/3	0.217	0.12-0.33
26 TR	3/3	0.232	0.14-0.52	1/1	0.49	--	3/3	0.858	0.65-1.2	3/3	0.521	0.33-0.74
27 SR	3/3	0.262	0.17-0.34	0/0	--	--	2/3	0.119	<0.11-0.28	3/3	0.194	0.18-0.22
		$\bar{X}$ = 0.218 BC			$\bar{X}$ = 0.420 AB			$\bar{X}$ = 0.303 A			$\bar{X}$ = 0.280 A	
SR												
28 SAC	3/3	0.680	0.32-1.3	3/3	0.812	0.47-1.4	3/3	0.348	0.31-0.40	1/3	0.397	<0.24-4.3
		$\bar{X}$ = 0.680 AB			$\bar{X}$ = 0.812 AB			$\bar{X}$ = 0.348 A			$\bar{X}$ = 0.397 A	
$f(df_1,df_2)^c$		8.90**			2.36*			2.72*			0.35	

[a] Chromium values for Sacramento blackfish at MSLBWA are N = 3/3, $\bar{X}$ = 2.28, and R = 1.8-3.0.

[b] Within a column, geometric grand means with the same capital letter are not significantly different (*P* >0.05, Tukey-Kramer hsd test).

[c] For bluegills, df1 = 7, df2 = 57; for common carp, df1 = 7, df2 = 40; for mosquitofish, df1 = 7, df2 = 76; for largemouth bass, df 1 = 5, df2 = 20, $^*P \le 0.05$, $^{**}P \le 0.01$.

Table 4. Mercury concentrations (µg/g dry weight) in five fishes from the Sacramento and San Joaquin valleys, September-December, 1986. N, sample size (no. detected/no. analyzed); $\bar{X}$, mean (geometric mean if N >1); R, range. Refer to Figure 1 for locations of collection sites[a].

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR1												
1 SJRFW	3/3	0.227	0.18-0.27	3/3	0.436	0.41-0.47	3/3	0.240	0.23-0.25	0/0	--	--
2 SJR145	3/3	0.217	0.15-0.26	1/1	0.21	--	3/3	0.235	0.22-0.25	0/0	--	--
	$\bar{X}$ = 0.222 BC			$\bar{X}$ = 0.364 AB			$\bar{X}$ = 0.237 AB			$\bar{X}$ = --		
SJR2												
3 SJRMP	3/3	0.144	0.13-0.18	0/0	--	--	3/3	0.167	0.14-0.19	0/0	--	--
4 SJRFB	3/3	0.255	0.23-0.28	3/3	0.459	0.37-0.64	3/3	0.331	0.32-0.35	0/0	--	--
5 SJR152	3/3	0.270	0.24-0.33	0/0	--	--	3/3	0.212	0.20-0.22	0/0	--	--
6 SJR165	3/3	0.326	0.29-0.35	2/2	0.505	0.37-0.70	3/3	0.246	0.24-0.26	3/3	0.687	0.50-0.81
	$\bar{X}$ = 0.238 B			$\bar{X}$ = 0.477 A			$\bar{X}$ = 0.232 AB			$\bar{X}$ = 0.687 A		
SJR3												
7 SJRFF	3/3	0.496	0.48-0.52	3/3	0.111	0.10-0.14	3/3	0.201	0.19-0.22	0/0	--	--
8 SJRHF	3/3	0.367	0.33-0.39	3/3	0.205	0.13-0.44	3/3	0.253	0.24-0.27	3/3	0.932	0.53-1.4
	$\bar{X}$ = 0.427 A			$\bar{X}$ = 0.151 B			$\bar{X}$ = 0.225 AB			$\bar{X}$ = 0.932 A		
SJR4												
9 SJRCL	3/3	0.384	0.31-0.45	3/3	0.289	0.25-0.33	3/3	0.293	0.28-0.30	0/0	--	--
10 SJRLP	3/3	0.424	0.36-0.48	2/2	0.327	0.32-0.34	3/3	0.258	0.25-0.26	0/0	--	--

Table 4. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR4 (Continued)												
11 SJR132	2/2	0.311	0.24-0.41	2/2	0.274	0.16-0.47	3/3	0.280	0.23-0.40	0/0	--	--
12 SJRDF	3/3	0.306	0.26-0.34	3/3	0.446	0.28-0.75	3/3	0.362	0.36-0.36	3/3	0.936	0.80-1.3
	$\bar{X}$ = 0.357 A			$\bar{X}$ = 0.334 AB			$\bar{X}$ = 0.296 A			$\bar{X}$ = 0.936 A		
TRIB1												
13 FS	3/3	0.130	0.11-0.15	0/0	--	--	3/3	0.148	0.12-0.18	0/0	--	--
14 DMC	0/0	--	--	0/0	--	--	3/3	0.147	0.15-0.15	0/0	--	--
15 OC	0/0	--	--	1/1	0.11	--	3/3	0.176	0.17-0.18	0/0	--	--
	$\bar{X}$ = 0.130 C			$\bar{X}$ = 0.11 AB			$\bar{X}$ = 0.157 B			$\bar{X}$ = --		
TRIB2												
16 MSLBWA	3/3	0.159	0.13-0.22	3/3	0.078	0.06-0.10	3/3	0.159	0.15-0.17	0/0	--	--
17 SSHR	0/0	--	--	0/0	--	--	3/3	0.274	0.26-0.28	0/0	--	--
18 SSSLNWR	2/2	0.273	0.27-0.27	3/3	0.275	0.13-0.45	3/3	0.198	0.18-0.23	3/3	0.349	0.22-0.53
19 C130	0/0	--	--	0/0	--	--	3/3	0.457	0.42-0.50	0/0	--	--
20 MSGCR	3/3	0.204	0.19-0.21	3/3	0.419	0.22-0.69	3/3	0.188	0.18-0.20	2/2	0.365	0.35-0.38
21 AC	3/3	0.299	0.26-0.36	3/3	0.488	0.34-0.80	3/3	0.429	0.42-0.45	0/0	--	--
22 HC	1/1	0.33	--	0/0	--	--	3/3	0.140	0.12-0.17	0/0	--	--
23 MC	0/0	--	--	0/0	--	--	3/3	0.239	0.22-0.26	0/0	--	--
24 LBC	3/3	0.227	0.17-0.35	3/3	0.144	0.10-0.18	3/3	0.214	0.21-0.22	0/0	--	--
	$\bar{X}$ = 0.230 B			$\bar{X}$ = 0.229 AB			$\bar{X}$ = 0.236 A			$\bar{X}$ = 0.355 A		

Table 4. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
TRIB3												
25 MR	3/3	0.379	0.36-0.39	3/3	0.538	0.36-0.71	3/3	0.151	0.12-0.17	3/3	0.456	0.45-0.46
26 TR	3/3	0.364	0.31-0.41	1/1	0.51	--	3/3	0.301	0.29-0.31	3/3	1.16	0.58-1.8
27 SR	3/3	0.354	0.31-0.38	0/0	--	--	3/3	0.254	0.25-0.27	3/3	1.17	0.52-1.9
	$\bar{X}$ = 0.366 A			$\bar{X}$ = 0.529 A			$\bar{X}$ = 0.226 AB			$\bar{X}$ = 0.852 A		
SR												
28 SAC	3/3	0.356	0.27-0.43	3/3	0.237	0.17-0.32	3/3	0.304	0.30-0.31	3/3	0.335	0.21-0.48
	$\bar{X}$ = 0.356 AB			$\bar{X}$ = 0.237 AB			$\bar{X}$ = 0.304 A			$\bar{X}$ = 0.335 A		
$f(df_1,df_2)^c$	11.67**			3.17**			3.96**			3.63*		

[a] Mercury values for Sacramento blackfish at MSLBWA are N = 3/3, $\bar{X}$ = 0.0593, and R = 0.052-0.068.

[b] Within a column, geometric grand means with the same capital letter are not significantly different (P >0.05, Tukey-Kramer hsd test.

[c] For bluegills, df1 = 7, df2 = 57; for common carp, df1 = 7, df2 = 40; for mosquitofish, df1 = 7, df2 = 76; for largemouth bass, df1 = 5, df2 = 20; *P ≤0.05, **P ≤0.01.

Table 5. Molybdenum concentrations (μg/g dry weight) in five fishes from the Sacramento and San Joaquin valleys, September-December, 1986. N, sample size (no. detected/no. analyzed); $\bar{X}$, mean (geometric mean if N >1); R, range. Refer to Figure 1 for locations of collection sites[a].

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}$	R	N	$\bar{X}$	R	N	$\bar{X}$	R	N	$\bar{X}$	R
SJR1												
2 SJR145	0/1	<0.26	--	0/1	<0.26	--	0/1	<0.26	--	0/0	--	--
		$\bar{X}$ = <0.26			$\bar{X}$ = <0.26			$\bar{X}$ = <0.26			$\bar{X}$ = --	
SJR2												
6 SJR165	1/1	0.35	--	1/1	0.59	--	1/1	0.70	--	1/1	0.29	--
		$\bar{X}$ = 0.35			$\bar{X}$ = 0.59			$\bar{X}$ = 0.70			$\bar{X}$ = 0.29	
SJR3												
7 SJRFF	1/1	0.42	--	0/1	<0.24	--	1/1	0.26	--	0/0	--	--
8 SJRHF	1/1	0.29	--	1/1	0.76	--	1/1	0.34	--	0/1	<0.28	--
		$\bar{X}$ = 0.346			$\bar{X}$ = 0.302			$\bar{X}$ = 0.298			$\bar{X}$ = <0.28	
TRIB2												
20 MSGCR	0/1	<0.26	--	1/1	1.0	--	0/1	<0.26	--	0/1	<0.26	--
		$\bar{X}$ = <0.26			$\bar{X}$ = 1.0			$\bar{X}$ = <0.26			$\bar{X}$ = <0.26	
TRIB3												
26 TR	0/0	--	--	1/1	0.73	--	1/1	0.24	--	1/1	0.48	--
		$\bar{X}$ = --			$\bar{X}$ = 0.73			$\bar{X}$ = 0.24			$\bar{X}$ = 0.48	

Table 5. (Continued)

Region, collection site,	Bluegills			Common carp			Mosquitofish			Largemouth bass		
and ANOVA	N	$\bar{X}$	R	N	$\bar{X}$	R	N	$\bar{X}$	R	N	$\bar{X}$	R
SR												
28 SAC	1/1	0.92	--	1/1	0.75	--	1/1	0.47	--	1/1	0.69	--
	$\bar{X}$ = 0.92			$\bar{X}$ = 0.75			$\bar{X}$ = 0.47			$\bar{X}$ = 0.69		
$f(df_1, df_2)$[c]	10.30			0.37			13.64			--		

[a] Molybdenum values for Sacramento blackfish at MSLBWA are N = 0/0, $\bar{X}$ = --, and R = --.

[b] Due to insufficient numbers of samples, statistical differences among grand geometric means could not be calculated by Tukey-Kramer hsd tests.

[c] For bluegills, df1 = 4, df2 = 1; for common carp, df1 = 5, df2 = 1; for mosquitofish, df1 = 6, df2 = 1; for largemouth bass, df1 = 4, df2 = 0; none of the f values were significant ($\underline{P} > 0.05$).

Table 6. Selenium concentrations (µg/g dry weight) in five fishes from the Sacramento and San Joaquin valleys, September-December, 1986. N, sample size (no. detected/no. analyzed) $\bar{X}$, mean (geometric mean if N >1); R, range. Refer to Figure 1 for locations of collection sites[a].

Region, collection site,	Bluegills			Common carp			Mosquitofish			Largemouth bass		
and ANOVA	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR1												
1 SJRFW	3/3	0.396	0.38-0.41	3/3	0.569	0.50-0.65	3/3	0.465	0.45-0.48	0/0	--	--
2 SJR145	3/3	0.528	0.52-0.53	1/1	0.56	--	3/3	0.600	0.59-0.63	0/0	--	--
	$\bar{X}$ = 0.457 D			$\bar{X}$ = 0.567 D			$\bar{X}$ = 0.528 E			$\bar{X}$ = --		
SJR2												
3 SJRMP	3/3	1.65	1.5 -1.7	0/0	--	--	3/3	1.76	1.5 -2.1	0/0	--	--
4 SJRFB	3/3	1.63	1.4 -1.9	3/3	2.50	2.2 -2.8	3/3	1.80	1.7 -1.9	0/0	--	--
5 SJR152	3/3	2.06	2.0 -2.1	0/0	--	--	3/3	1.90	1.8 -2.0	0/0	--	--
6 SJR165	5/5	1.18	1.1 -1.7	2/2	0.900	0.56-1.5	3/3	0.944	0.91-0.98	3/3	0.919	0.88-0.94
	$\bar{X}$ = 1.53 B			$\bar{X}$ = 1.66 BC			$\bar{X}$ = 1.54 BCD			$\bar{X}$ = 0.919 C		
SJR3												
7 SJRFF	3/3	1.48	1.2 -1.7	3/3	6.29	5.9 -6.9	3/3	3.84	3.7 -4.0	0/0	--	--
8 SJRHF	7/7	2.10	1.8 -2.4	3/3	2.64	2.2 -3.0	3/3	2.08	2.0 -2.1	3/3	1.89	1.7 -2.0
	$\bar{X}$ = 1.89 B			$\bar{X}$ = 4.07 AB			$\bar{X}$ = 2.82 AB			$\bar{X}$ = 1.89 B		
SJR4												
9 SJRCL	3/3	1.99	1.9 -2.1	3/3	2.18	1.9 -2.5	3/3	1.64	1.6 -1.7	0/0	--	--
10 SJRLP	3/3	1.64	1.5 -1.7	2/2	1.65	1.3 -2.0	3/3	1.69	1.6 -1.8	0/0	--	--

Table 6. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
TRIB3												
25 MR	3/3	1.06	1.0 -1.1	3/3	1.48	1.3-1.7	3/3	1.00	0.66-1.4	3/3	1.06	0.98-1.2
26 TR	3/3	1.01	0.98-1.0	1/1	1.6	--	3/3	0.997	0.98-1.0	3/3	0.965	0.95-1.1
27 SR	3/3	0.811	0.76-0.85	0/0	--	--	3/3	0.789	0.77-0.80	3/3	1.02	0.90-1.1
	$\bar{X}$ = 0.954 C			$\bar{X}$ = 1.51 CD			$\bar{X}$ = 0.924 DE			$\bar{X}$ = 1.02 C		
SR												
28 SAC	3/3	1.83	1.7-2.1	3/3	1.35	1.2-1.5	3/3	1.46	1.4-1.5	3/3	1.22	1.1-1.3
	$\bar{X}$ = 1.83 B			$\bar{X}$ = 1.35 CD			$\bar{X}$ = 1.46 BCD			$\bar{X}$ = 1.22 BC		
$f(df_1,df_2)^d$	69.93**			12.31**			24.83**			23.13**		

[a] Selenium values for Sacramento blackfish at MSLBWA are N = 3/3, $\bar{X}$ = 3.87, and R = 3.7-4.1.

[b] Within a column, geometric grand means with the same capital letter are not significantly different ($\underline{P}$ >0.05, Tukey-Kramer hsd test.

[c] One more bluegill composite sample was not included due to the absence of gonad selenium data.

[d] For bluegills, df1 = 7, df2 = 72; for common carp, df1 = 7, df2 = 40; for mosquitofish, df1 = 7, df2 = 76; for largemouth bass, df1 = 5, df2 = 20; **$\underline{P}$ ≤0.01.

Table 6. (Continued)

Region, collection site, and ANOVA	Bluegills			Common carp			Mosquitofish			Largemouth bass		
	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R	N	$\bar{X}^b$	R
SJR4 (Continued)												
11 SJR132	2/2	1.50	1.3 -1.7	2/2	1.68	1.7 -1.7	3/3	1.51	1.5 -1.6	0/0	--	--
12 SJRDF	6/6^b	1.50	1.3 -1.7	3/3	1.51	1.4 -1.8	3/3	1.61	1.6 -1.7	3/3	1.38	1.3-1.5
	$\bar{X}$ = 1.62 B			$\bar{X}$ = 1.75 C			$\bar{X}$ = 1.61 BC			$\bar{X}$ = 1.38 BC		
TRIB 1												
13 FS	3/3	1.25	1.2 -1.3	0/0	--	--	3/3	1.31	1.1-1.5	0/0	--	--
14 DMC	0/0	--	--	0/0	--	--	3/3	1.05	0.96-1.1	0/0	--	--
15 OC	0/0	--	--	1/1	1.3	--	3/3	1.78	1.7-1.8	0/0	--	--
	$\bar{X}$ = 1.25 BC			$\bar{X}$ = 1.3 ACD			$\bar{X}$ = 1.35 CD			$\bar{X}$ = --		
TRIB2												
16 MSLBWA	7/7	5.62	5.1 -6.7	3/3	6.18	6.1-6.4	3/3	5.66	5.5-5.9	0/0	--	--
17 SSHR	0/0	--	--	0/0	--	--	3/3	1.61	1.6-1.7	0/0	--	--
18 SSSLNWR	2/2	2.94	2.9 -3.0	3/3	3.05	2.7-3.5	3/3	3.28	3.2-3.5	3/3	3.05	2.8-3.4
19 C130	0/0	--	--	0/0	--	--	3/3	5.38	5.1-5.7	0/0	--	--
20 MSGCR	5/5	8.24	6.2 -9.4	3/3	8.41	6.0-10.	3/3	10.8	10.-11.	2/2	9.03	8.4-9.7
21 AC	3/3	7.37	6.1 -8.8	3/3	4.77	2.2-7.1	3/3	3.46	3.4-3.5	0/0	--	--
22 HC	1/1	8.5	--	0/0	--	--	3/3	1.90	1.8-2.0	0/0	--	--
23 MC	0/0	--	--	0/0	--	--	3/3	3.09	2.9-3.3	0/0	--	--
24 LBC	3/3	2.53	1.9 -3.4	3/3	1.91	1.7-2.3	3/3	1.94	1.9-2.0	0/0	--	--
	$\bar{X}$ = 5.47 A			$\bar{X}$ = 4.28 A			$\bar{X}$ = 3.44 A			$\bar{X}$ = 4.71 A		

Table 7. Relations between the concentrations (µg/g dry weight) of arsenic, mercury, and selenium in whole-body samples of bluegills (BG) and largemouth bass (LMB) with those in their skinless fillets. Concentrations in skinless fillets (SF) can be estimated from concentrations in the whole body (WB) by the formula, SF = $\underline{a} + \underline{b}$ WB.[a]

Element	Species	Intercept (a)	Slope (b)	N	R^2
Arsenic	BG	-0.063	0.964	15	0.924
	LMB	-0.024	1.126	11	0.738
Mercury	BG	-0.127	2.039	11	0.983
Selenium	BG	0.045	1.227	15	0.982
	LMB	-0.388	1.322	11	0.996

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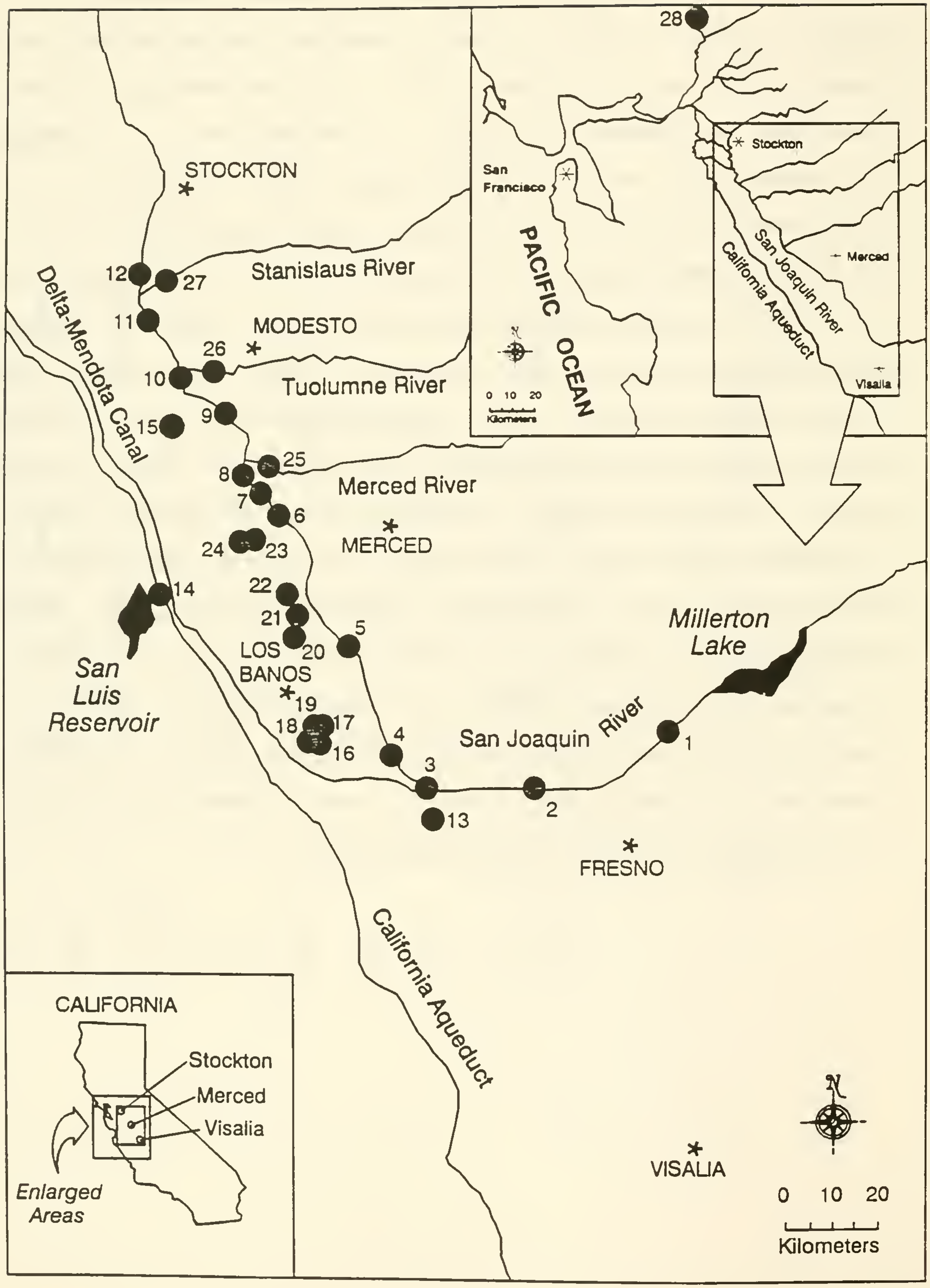
[a] Formulas for boron, chromium, and molybdenum were omitted because concentrations in skinless fillets and whole bodies were not significantly correlated ($\underline{P} > 0.05$).

Figure 1. The study area, showing the general locations of sampling sites. Sampling sites in regions that subdivide the San Joaquin River are as follows: SJR1, (1) Fort Washington Beach Park, and (2) Highway 145; SJR2, (3) Mendota Pool, (4) Firebaugh, (5) Highway 152, and (6) Lander Avenue; SJR3, (7) Fremont Ford State Recreation Area and (8) above Hills Ferry Road; and SJR4, (9) Crows Landing Road, (10) Laird County Park, (11) Maze Road, and (12) Durham Ferry State Recreation Area. Sampling sites in the remaining regions are as follows: TRIB1, (13) Fresno Slough at the Mendota Wildlife Area, (14) Delta-Mendota Canal at O'Neill Forebay, and (15) Orestimba Creek at Highway 33; TRIB2, (16) Helm Canal, (17) Agatha Canal, (18) Main Canal, (19) Camp 13 Ditch, (20) Mud Slough at Los Banos Wildlife Area, (21) Salt Slough at Hereford Road, (22) Salt Slough at the San Luis National Wildlife Refuge, (23) Mud Slough at Gun Club Road, and (24) Los Banos Creek at Gun Club Road; TRIB3, (25) Merced River at George J. Hatfield State Recreation Area, (26) Tuolumne River at Shiloh Road, and (27) Stanislaus River at Caswell State Park; and SR, (28) Sacramento River at Knights Landing.


28
Stockton
San Francisco
PACIFIC OCEAN
San Joaquin River
California Aqueduct
Merced
Visalia
0 10 20
Kilometers
STOCKTON
12
27
Stanislaus River
Delta-Mendota Canal
11
MODESTO
26
10
Tuolumne River
9
15
25
8
Merced River
7
6
MERCED
24
23
22
14
21
20
5
LOS BANOS
San Luis Reservoir
19
18
17
16
4
3
San Joaquin River
1
Millerton Lake
2
13
FRESNO
California Aqueduct
CALIFORNIA
Stockton
Merced
Visalia
Enlarged Areas
VISALIA
0 10 20
Kilometers

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SELENIUM IN FRESHWATER

Introduction

Contamination of Kesterson Reservoir and adjacent wetlands by selenium-laden irrigation drainage water has caused concern for potential adverse effects on fish and wildlife resources. Elevated water concentrations of selenium in subsurface drainage water continues to enter freshwater habitats of the Grasslands area located south and adjacent to Kesterson. Selenium-laden water enters the Grasslands by way of the Helm and Main canals and from smaller ditches and drains; water leaves the Grasslands through Mud (north) and Salt sloughs, which serve as the primary drainage system, and in turn, drains into the San Joaquin River. A second route of selenium entry into the San Joaquin River is by subsurface seepage from Kesterson Reservoir, which is located adjacent to the river.

Entry of elevated concentrations of selenium into the San Joaquin River could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily during the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to selenium in water qualities that simulate potential environmental conditions in freshwater. Acute and chronic toxicity studies were conducted with various forms of selenium, individually and in various mixtures that simulate reported environmental ratios.

Methods

Acute toxicity tests: The swimup life stage (0.5 g) of fall-run chinook salmon was used in 15 acute toxicity studies to determine the 96-hour LC50 values (i.e., the concentration that kills 50% of the test animals in a 96-hour time period). Fish were obtained as eyed eggs from either the Merced River Fish Facility, Snelling, CA, or Iron Gate Salmon and Steelhead Hatchery, Hornbrook, CA. The forms of selenium tested individually were selenate, selenite, and selenomethionine, and the environmental ratios of selenate:selenite tested were 3.5:1, 6:1, and 14:1. Acute toxicity tests were conducted in accordance with accepted procedures from the U.S. Environmental Protection Agency and American Society for Testing and Materials. One deviation from those procedures in our studies was the use of standardized test waters that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 37-fold in a standardized fresh receiving water. Actual drainage water was not used in toxicity studies because its water quality varies with location and time of year. The water quality of the test water, prior to addition of test chemicals, was pH 7.8, 211 ppm hardness, 87 ppm alkalinity as HCO_3, 46 ppm calcium, 24 ppm magnesium, 73 ppm sodium, 59 ppm chloride, and 185 ppm sulfate. This reconstituted test water was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate.

In addition, several life stages of chinook salmon were tested with selenate and selenite to determine the sensitivity of these life stages to two forms of selenium. The LC50 values were calculated with the Litchfield-Wilcoxon method.

Chronic toxicity test: The swimup life stage (0.5 g) of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to waterborne selenium. The forms of selenium tested were sodium selenate and sodium selenite, and the environmental ratio of selenate:selenite tested was 6:1. The chronic toxicity test was conducted in a similar standardized test water as used in acute toxicity tests. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted test water to each exposure aquarium at 15-minute intervals. Six duplicate nominal concentrations of selenium were tested (0, 8, 17, 35, 70, and 140 ppb). In each exposure aquarium throughout the study, mortality was recorded daily, fish behavior checklist evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and at 30-day intervals, fish samples were collected for measurement of selenium concentrations, fish length and weight were measured, fish behavior was videotaped, and fish swimming performance was measured. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 20 fish from each treatment following the 120-day exposure.

Water samples from the chronic toxicity study were analyzed for selenium concentrations at the Yankton FRS, SD. Tissue samples from the chronic toxicity study were analyzed for selenium concentrations at NFCR-Columbia (MO).

Principal Findings

Acute toxicity tests: Acute toxicity tests with early life stages of chinook salmon indicate that swimup fry were more sensitive to selenate and selenite than were the eyed-egg or alevin life stages (Table 1). Acute toxicity tests with two stocks of young chinook salmon from California tested in a reconstituted freshwater revealed no difference in their sensitivity to selenite and selenate (Table 2). No definitive tests with selenomethionine were completed because of the prohibitive costs of conducting further tests. The 96-hour LC50 value of the 14:1 selenium mixture was greater than that of the 3.5:1 and 6:1 mixtures but there was no difference in toxicity between the 6:1 and 3.5:1 mixtures.

The hazard potential of selenate and selenite to indigenous salmon populations was estimated by comparing the 96-hour LC50 values with the expected environmental concentrations in the San Luis Drain, which have averaged about 0.35 ppm total selenium. Based on the pooled 96-hour LC50 values for chinook salmon and assuming the ratio of selenate to selenite in drainage water is 6:1 (0.30 ppm selenate and 0.05 ppm selenite), the calculated margins of safety for selenate in freshwater was 383 and for selenite it was 276. However, the margin of safety for selenium as a 6:1 mixture of selenate and selenite in freshwater was 145. Safety margins less than 100 indicate high potential for adverse effects on aquatic resources, thus, the concentrations of selenate and selenite found in the San Luis Drain -- when tested individually or in a 6:1 mixture in freshwater but without other inorganics -- do not seem to pose a threat to the health and well-being of chinook salmon from a waterborne route of exposure. However, the toxicity of these selenium forms to fish may change in the presence of other waterborne inorganic contaminants found in subsurface irrigation drainage water, or if consumed in selenium-laden food organisms via a dietary route of exposure. Also, it is possible that the waterborne inorganic selenium could be biologically converted into dissolved organic forms that are much more toxic.

Chronic toxicity test: Preliminary results from the 120-day chronic toxicity test indicate that waterborne exposure of young chinook salmon to measured concentrations of selenium as high as 139 ppb had no effect on their survival, growth (Table 3), or whole-body residues

(Table 4). The low whole-body concentrations of selenium in fish after 120 days of exposure are below the concentrations shown in other research with chinook salmon to cause effects on survival and growth.

There was no effect on survival of fish from the 10-day seawater challenge test.

Work Remaining

The results of the chronic toxicity study with the swimup life stage of chinook salmon exposed to a mixture of selenate and selenite (6:1 ratio) are awaiting further statistical analyses and analyses of fish behavior and swimming performance measurements.

Research Needs

Because no definitive toxicity tests were accomplished with selenomethionine, future research should be conducted to determine its toxicity to two life stages of chinook salmon.

The lack of effects in the chronic toxicity test do not correspond with the results of other research with chinook salmon or trout exposed to selenium and therefore the waterborne selenium test should be repeated.

Literature Generated From These Studies

Manuscript

Hamilton, S.J., and K.J. Buhl. 1990. Acute toxicity of boron, molybdenum, and selenium to fry of chinook salmon and coho salmon. Archives of Environmental Contamination and Toxicology 19: 366-373.

In-house report

U.S. Fish and Wildlife Service Research Information Bulletin #89-47: Young salmon may be adversely affected by boron and selenium.

Table 1. Acute toxicity (LC50s in ppm; 95% confidence intervals in parentheses) of selenite and selenate to different life stages of chinook salmon in soft water.

Chemical	Life stage or weight (g)	Exposure period (hour)	
		24	96
Selenite	eyed egg	>560	>560
	alevin	202	104
		(151-270)	(87-124)
	0.31	100	27.3
		(84-118)	(19.2-38.7)
	0.46	65.8	13.1
		(0.5-85.6)	(10.8-16.0)
Selenate	eyed egg	>1000	>1000
	alevin	>320	>320
	0.31	511	114
		(381-686)	(85-154)

Table 2. Acute toxicity (LC50s in ppm: 95% confidence intervals in parentheses) of selenite and selenate to different stocks of chinook salmon from California tested in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water. One stock of chinook salmon tested was from the Merced River Fish Facility (MR) and the other was from Iron Gate Salmon and Steelhead Hatchery (IG). Also given is the pooled (P) acute toxicity values for the combined data from the two stocks of chinook salmon.

Chemical	Stock	Mean weight (g)	Exposure period (hour)	
			24	96
Selenite	MR	0.5	46.1 (38.2-55.6)	14.8 (11.4-19.1)
	IG	0.7	57.0 (48.3-67.1)	13.0 (8.6-19.5)
	P		46.9 (38.2-57.5)	13.8 (10.8-17.6)
Selenate	MR	0.5	464 (360-600)	121 (86-171)
	IG	0.7	510 (361-721)	100 (74-135)
	P		475 (421-537)	115 (94-141)
Selenomethionine	MR	0.5	>21.6	>21.6
Selenate:Selenite Mixtures				
3.5:1	MR	0.5	145 (113-186)	46.6 (35.8-60.7)
6:1	MR	0.5	189 (157-228)	50.9 (39.3-65.8)
14:1	MR	0.5	318 (242-418)	85.5 (66.5-110)

Table 3. Mean and standard error (in parentheses) of length (mm) and weight (g) of chinook salmon exposed to a 6:1 mixture of selenate and selenite for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water. No significant differences from control at P≤0.05.

Days of exposure	Measured selenium exposure concentration (ppb)					
	<2[a]	9	17	35	63	139
Length						
30	41.4 (0.4)	43.2 (0.4)	42.7 (0.4)	43.3 (0.5)	43.6 (0.4)	43.2 (0.5)
60	51.0 (1.1)	56.5 (1.0)	54.4 (1.0)	56.3 (0.9)	54.7 (1.1)	54.8 (1.2)
90	64.6 (1.7)	70.6 (1.2)	68.8 (1.0)	70.1 (1.1)	68.1 (1.3)	68.6 (1.4)
120	74.9 (2.1)	80.9 (1.7)	77.8 (1.2)	80.7 (1.3)	77.6 (1.4)	80.0 (1.4)
Weight						
30	0.55 (0.02)	0.65 (0.03)	0.61 (0.02)	0.66 (0.03)	0.65 (0.03)	0.64 (0.03)
60	1.10 (0.08)	1.49 (0.07)	1.33 (0.07)	1.46 (0.07)	1.36 (0.08)	1.40 (0.09)
90	2.39 (0.18)	3.05 (0.15)	2.82 (0.13)	3.03 (0.14)	2.79 (0.16)	2.94 (0.17)
120	3.68 (0.30)	4.67 (0.30)	4.09 (0.20)	4.58 (0.24)	4.09 (0.21)	4.52 (0.25)

[a] Control treatment.

Table 4. Concentrations of selenium (ppm dry weight) in whole-body of chinook salmon exposed to a 6:1 mixture of selenate and selenite for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water.

Days of exposure	Measured selenium exposure concentration (ppb)					
	<2[a]	9	17	35	63	139
30	2.06	2.07	2.16	3.26	3.23	3.53
60	1.56	1.51	1.70	2.17	2.44	2.39
90	1.42	1.36	1.44	1.54	1.86	1.88
120	0.98	1.12	1.22	1.32	1.28	1.84

[a] Control treatment.

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SELENIUM IN BRACKISH WATER

Introduction

Originally the San Luis Drain was planned to terminate near Chipps Island in the western Sacramento-San Joaquin Delta, but Federal budget restrictions and environmental and political concerns halted construction of the drain in 1975 at the site of the flow-regulating Kesterson Reservoir. In 1979, the Interagency Drainage Program recommended completion of the San Luis Drain to the Delta, which would directly deliver irrigation return flows to a brackish receiving water. The Delta already receives irrigation return water from the northern portion of the federal service area that drains into the middle reaches of the San Joaquin River.

Entry of elevated concentrations of selenium into the Delta could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily in the pre-smolt life stage when advanced fry are inhabiting the Delta prior to smoltification and seaward migration.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to selenium in water qualities that simulate potential environmental conditions in brackish water. Acute and chronic toxicity studies were conducted with various forms of selenium individually and in various mixtures to simulate reported environmental ratios.

Methods

Acute toxicity tests: The advanced fry life stage (2 g) of fall-run chinook salmon was used in eight acute toxicity studies to determine the 96-hour LC50 values (i.e., concentration that kills 50% of the test animals in a 96-hour time period). Fish were obtained as eyed eggs from either the Merced River Fish Facility, Snelling, CA, or Iron Gate Salmon and Steelhead Hatchery, Hornbrook, CA. The forms of selenium tested individually were selenate, selenite, and selenomethionine, and the environmental mixtures of selenate:selenite tested were 3.5:1, 6:1, and 14:1. Acute toxicity tests were conducted in accordance with accepted procedures from the U.S. Environmental Protection Agency and American Society for Testing and Materials. One deviation from those procedures in our studies was the use of standardized test waters that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 22.5-fold in a standardized brackish receiving water (about 1.2 ppthousand salinity). Actual drainage water was not used in toxicity studies because its water quality varies with location and time of year. The water quality of the test water, prior to addition of test chemicals, was pH 7.8, 343 ppm hardness, 37 ppm alkalinity as HCO_3, 39 ppm calcium, 60 ppm magnesium, 469 ppm sodium, 724 ppm chloride, and 288 ppm sulfate. This reconstituted test water was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with Instant Ocean and small amounts of various other salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. The LC50 values were calculated with the Litchfield-Wilcoxon method.

Chronic toxicity test: The advanced fry life stage of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to waterborne selenium. The forms of selenium tested were sodium selenate and sodium selenite, and the environmental ratio of selenate:selenite tested was 6:1. The chronic toxicity test was conducted in a similar standardized test water as used in acute toxicity tests. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted test water to each exposure aquarium at 15-minute intervals. Six duplicate nominal concentrations of selenium were tested (0, 8, 17, 35, 70, 140, and 280 ppb). In each exposure aquarium throughout the study, mortality was recorded daily, fish behavior evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and at 30-day intervals, fish samples were collected for measurement of selenium concentrations, fish length and weight were measured, fish behavior was videotaped, and fish swimming performance was measured. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 20 fish from each treatment following the 120-day exposure.

Water samples from the chronic toxicity study were analyzed for selenium concentrations at the Yankton FRS, SD. Tissue samples from the chronic toxicity study were analyzed for selenium concentrations at NFCR-Columbia (MO).

Principal Findings

Actue toxicity tests: Acute toxicity tests with two stocks of young chinook salmon from California tested in a reconstituted brackish water revealed no difference in their sensitivity to selenite and selenate (Table 1). No definitive tests with selenomethionine were completed because of the prohibitive costs of conducting further tests. The 96-hour LC50 value of the 3.5:1 selenium mixture was less than that of the 6:1 mixture but not the 14:1 mixture; there was no difference in toxicity between the 6:1 and 14:1 mixtures.

The hazard potential of selenate and selenite to indigenous salmon populations was estimated by comparing the 96-hour LC50 values with the expected environmental concentrations in the San Luis Drain, which have averaged about 0.35 ppm total selenium. Based on the pooled 96-hour LC50 values for chinook salmon and assuming the ratio of selenate to selenite in drainage water is 6:1 (0.30 ppm selenate and 0.05 ppm selenite), the calculated margins of safety for selenate in brackish water was 479 and for selenite it was 468. However, the margin of safety for selenium as a 6:1 mixture of selenate and selenite in brackish water was 220. Safety margins less than 100 indicate high potential for adverse effects on aquatic resources, thus, the concentrations of selenate and selenite found in the San Luis Drain -- when tested individually or in a 6:1 mixture in brackish water but without other inorganics -- do not seem to pose a threat to the health and well-being of chinook salmon from a waterborne route of exposure. However, the toxicity of these selenium forms to fish may change in the presence of other waterborne inorganic contaminants found in subsurface irrigation drainage water or if consumed in selenium-laden food organisms via a dietary route of exposure. Also, waterborne inorganic selenium can be biologically converted to dissolved organic forms that may be more toxic.

Chronic toxicity test: Preliminary results from the 120-day chronic toxicity test indicate that waterborne exposure of young chinook salmon to 282 ppb of selenium caused significant reductions in growth of fish after 90 days of exposure (Table 2), and 100% mortality after 100 days of exposure. Concentrations of selenium in whole-body of exposed salmon are given in Table 3.

There was no effect of prior exposure to selenium for 120 days (up to 282 ppb) on the survival of fish in the 10-day seawater challenge test.

Work Remaining

The results of the chronic toxicity study with the advanced fry life stage of chinook salmon exposed to a mixture of selenate and selenite (6:1 ratio) are awaiting further statistical analyses and analyses of fish behavior and swimming performance measurements.

Research Needs

Because no definitive toxicity tests were accomplished with selenomethionine, future research should be conducted to determine its toxicity to two life stages of chinook salmon.

Literature Generated From These Studies

Manuscript

Hamilton, S.J., and K.J. Buhl. 1990. Acute toxicity of boron, molybdenum, and selenium to fry of chinook salmon and coho salmon. Archives of Environmental Contamination and Toxicology 19: 366-373.

In-house report

U.S. Fish and Wildlife Service Research Information Bulletin #89-47: Young salmon may be adversely affected by boron and selenium.

Table 1. Acute toxicity (LC50s in ppm; 95% confidence intervals in parentheses) of selenite and selenate to different stocks of chinook salmon from California tested in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water. One stock of chinook salmon tested was from the Merced River Fish Facility (MR) and the other was from Iron Gate Salmon and Steelhead Hatchery (IG). Also given is the pooled (P) acute toxicity values for the combined data from the two stocks of chinook salmon.

Chemical	Stock	Mean weight (g)	Exposure period (hour)	
			24	96
Selenite	MR	1.6	75.9 (63.8-90.2)	23.4 (17.2-31.8)
	IG	1.6	56.6 (39.6-80.7)	23.1 (16.6-32.1)
	P		65.6 (52.3-82.3)	23.4 (18.8-29.1)
Selenate	MR	1.6	316 (236-424)	134 (94-192)
	IG	1.6	>600	180 (136-239)
	P		484 (374-626)	149 (116-191)
Selenomethionine	MR	2.6	>21.6	>21.6
Selenate:Selenite Mixtures				
3.5:1	MR	2.4	240 (173-242)	51.9 (43.2-62.4)
6:1	MR	2.4	278	76.9 (65.4-90.5)
14:1	MR	2.4	277 (205-375)	67.0 (50.1-89.8)

Table 2. Mean and standard error (in parentheses) of length (mm) and weight (g) of chinook salmon exposed to a 6:1 mixture of selenate and selenite for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water. Astericks denote significant differences from the control (*P≤0.10).

Days of exposure	Measured selenium exposure concentration (ppb)					
	1[a]	12	34	63	133	282
Length						
30	107 (1)	106 (1)	107 (1)	107 (1)	108 (1)	108 (1)
60	121 (1)	119 (1)	121 (1)	120 (1)	120 (1)	122 (2)
90	135 (1)	131 (1)	135 (1)	135 (1)	135 (2)	129 (3)*
120	149 (2)	144 (2)	150 (2)	150 (1)	147 (2)	-
Weight						
30	10.5 (0.3)	10.0 (0.2)	10.3 (0.3)	10.1 (0.3)	10.6 (0.3)	10.5 (0.3)
60	15.9 (0.6)	14.9 (0.4)	16.0 (0.5)	15.6 (0.5)	15.5 (0.6)	15.9 (0.6)
90	23.3 (0.9)	20.6 (0.6)	22.7 (0.7)	22.8 (0.8)	22.1 (0.8)	19.7 (1.5)*
120	32.8 (1.3)	29.4 (1.0)	32.5 (1.4)	32.3 (1.0)	30.0 (1.2)*	-

[a] Control treatment.

Table 3. Concentrations of selenium (ppm dry weight) in whole-body of chinook salmon exposed to a 6:1 mixture of selenate and selenite for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water.

Days of exposure	Measured selenium exposure concentration (ppb)					
	1[a]	12	34	63	133	282
30	1.09	1.17	1.16	1.18	1.26	1.65
60	1.12	1.32	1.18	1.20	1.23	1.42
90	1.11	1.11	1.11	1.14	1.24	1.50
120	1.14	1.10	2.28	1.25	4.15	-

[a] Control treatment.

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SAN LUIS DRAIN CONTAMINANTS IN FRESHWATER

Introduction

Elevated concentrations of several inorganics were found in surface waters at 14 sites in the San Joaquin Valley including Kesterson Reservoir, the San Luis Drain collector lines, and a number of drainage canals between Mendota and Gustine, CA (Shelton and Miller, 1988). Even though selenium has been identified as the most obvious contaminant of concern in these water samples, significant concentrations of boron, chromium, iron, lead, molybdenum, vanadium, and zinc were also found in the samples. Contamination of aquatic habitats, i.e., wetlands, sloughs, and rivers, with elevated concentrations of inorganics could cause adverse effects on fish and wildlife. Subsurface drainage water contaminated with inorganics continues to enter freshwater habitats of the Grasslands area, located south and adjacent to Kesterson, by way of large canals, drains, and small ditches. This water leaves the Grasslands through Mud (north) and Salt sloughs, which serve as the primary drainage system, and in turn, drains into the San Joaquin River. Subsurface seepage from wetlands adjacent to the San Joaquin River such as Kesterson Reservoir is also another route of entry of contaminants to the river.

Entry of elevated concentrations of inorganics into the San Joaquin River could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily in the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to inorganics in water qualities that simulate potential environmental conditions in freshwater. Acute toxicity studies were conducted with arsenate, arsenite, boron, cadmium, chromate, copper, mercury, molybdenum, vanadium, and zinc, tested individually, and with 13-inorganic and 15-inorganic mixtures; one chronic toxicity study was conducted with a 16-inorganic mixture.

Methods

Acute toxicity tests: The swimup life stage (0.5 g) of fall-run chinook salmon was used in 13 acute toxicity studies to determine the 96-hour LC50 values (i.e., concentration that kills 50% of the test animals in a 96-hour time period). The inorganics tested individually were arsenate, arsenite, boron, cadmium, chromate, copper, mercury, molybdenum, vanadium, and zinc. Arsenate toxicity was determined in a pH-buffered test and in a non-pH-buffered test because the form of arsenate used in toxicity tests (arsenic(V)oxide hydrate) caused a pH shift during the test, which ranged from pH 3.0 to 6.9 depending on the treatment. The pH of the buffered test was adjusted to pH 7.0 with a sodium hydroxide-sodium phosphate mixture. Tests with mixtures of inorganics included those given above (except arsenite) plus lead, manganese, nickel, selenate, selenite, and silver. Mixtures were formulated to simulate the environmental ratios in standardized San Luis Drain water.

Acute toxicity tests were conducted in accordance with accepted procedures from the U.S. Environmental Protection Agency and American Society for Testing and Materials. One deviation from those procedures in our studies was the use of standardized test waters that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 37-fold in a standardized fresh receiving water. Actual drainage water was not used in toxicity studies because its water quality varies with location and time of year. The water quality of the test water, prior to addition of test chemicals, was pH 7.8, 211 ppm hardness, 87 ppm alkalinity as HCO_3, 46 ppm calcium, 24 ppm magnesium, 73 ppm sodium, 59 ppm chloride, and 185 ppm sulfate. This reconstituted test water was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various other salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. The LC50 values were calculated with the Litchfield-Wilcoxon method.

Chronic toxicity test: The swimup life stage (0.5 g) of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to a waterborne mixture of inorganics in the environmental ratios found in the San Luis Drain. The mixture of inorganics tested included arsenate, boron, cadmium, chromate, copper, ferric iron, lead, manganese, mercury, molybdenum, nickel, selenate, selenite, silver, vanadium, and zinc. The chronic toxicity test was conducted in a similar standardized test water as used in acute toxicity tests. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted water to each exposure aquarium at 15-minute intervals. Six duplicate nominal concentrations of the inorganic mixture were tested (Table 1). In each exposure aquarium throughout the study, mortality was recorded daily, fish behavior evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selected inorganic concentrations, and at 30-day intervals, fish samples were collected for measurement of selected inorganic concentrations, fish length and weight were measured, fish behavior was videotaped, and fish swimming performance was measured. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure.

Water samples from the chronic toxicity study were analyzed for concentrations of cadmium, copper, and selenium at the Yankton FRS, SD. Tissue samples from the chronic toxicity study were analyzed for concentrations of cadmium, copper, selenium, and zinc at NFCR-Columbia (MO).

Principal Findings

Acute toxicity tests: Toxicity to young chinook salmon of the inorganics tested varied considerably (Table 2) and is summarized in the following order of toxicity (selenium forms are included for comparison): cadmium (most toxic) > copper = mercury > > zinc > > selenite = vanadium > arsenite > arsenate (unbuffered) = selenate = chromate > arsenate buffered > > boron ? molybdenum (least toxic). In these tests, four of the 10 inorganics had 96-hour LC50 values that were less than a factor of 100 of their concentrations in undiluted San Luis Drain water (boron, cadmium, copper, and zinc). In general, a factor of 100-fold difference between the 96-hour LC50 value and the expected environmental concentration is the minimal safety margin for protection of aquatic organisms against sublethal toxicity for most inorganics. Consequently, elevated concentrations of boron, cadmium, copper, and zinc in slightly diluted San Luis Drain water would cause toxic effects in aquatic organisms in a fresh receiving water.

The acute toxicity of a mixture of 13 inorganics (arsenate, boron, cadmium, chromate, copper, lead, manganese, mercury, molybdenum, nickel, silver, vanadium, and zinc) in their environmental ratios found in standardized San Luis Drain water was equal in its toxicity to young chinook salmon tested in freshwater as when the 13-inorganic mixture contained selenate and selenite (Table 3). This result suggests that the waterborne toxicity of the mixture of inorganics was not dependent on the presence of selenate or selenite but rather on the joint toxicity of the other inorganic components of the mixture. Based on an evaluation of toxic units (a toxic unit is the ratio of the LC50 concentration of an inorganic in a mixture to the LC50 concentration of an inorganic tested individually), the major toxic component of the inorganic mixture was copper (toxic unit 0.488-0.510) followed closely by cadmium and boron (Table 3). Selenate and selenite had relatively low toxic unit values thus indicating their small contribution to the waterborne toxicity of the mixture of inorganics that was tested in their environmental ratios found in standardized San Luis Drain water.

Both the 13-inorganic mixture with and without selenate and selenite showed additive toxicity characteristics rather than antagonistic or synergistic characteristics because the values for the additive toxicity index overlapped zero (Table 3).

The hazard potential of the inorganic mixture with and without selenate and selenite to salmon populations was estimated by comparing the acute toxicity values with the expected environmental concentrations in the San Luis Drain. Based on the joint acute toxicity of the environmental mixture of inorganics, the calculated margin of safety for the mixture without selenium was 5.32, whereas for the mixture with selenium it was 5.56. Safety margins less than 100 indicate a high potential for adverse effects on aquatic resources, thus, the results of these acute toxicity tests indicate that the mixture of inorganics found in the San Luis Drain pose a "waterborne threat" to the health and well-being of young chinook salmon in freshwater regardless of whether selenium is present in the mixture or not.

Chronic toxicity test: Preliminary results from the 120-day chronic toxicity study indicate that waterborne exposure to nominal concentrations of the mixture of 16 inorganics in the high concentration treatment (Table 1) for 120 days had no effect on survival or growth of chinook salmon (Table 4). Analysis of concentrations of cadmium, copper, selenium, and zinc in tissue samples revealed no significant accumulation of these elements in fish exposed to the mixture for 120 days (Table 5). There was no effect on survival of fish from the 10-day seawater challenge test. The high treatment concentration of the inorganic mixture tested in the chronic toxicity study was about 14 times lower than the 96-hour LC50 value for the mixture that included selenium in one acute toxicity test. Consequently, exposure of fish for 120 days to the high treatment should have caused significant mortality and it is unclear why it did not.

Work Remaining

The results of the chronic toxicity study with the swimup life stage of chinook salmon exposed to a mixture of 16 inorganics are awaiting further statistical analyses and analyses of fish behavior and swimming performance measurements.

Research Needs

Because no definitive tests were accomplished with molybdenum, future research should be conducted to determine its toxicity to two life stages of chinook salmon.

The lack of effects in the chronic toxicity test does not correspond with the results of the acute toxicity test with the 15-inorganic mixture and therefore the acute toxicity test should be repeated. The only difference between the tests was the inclusion of ferric iron in the mixture used in the chronic toxicity test but not in the acute test. It is possible that the iron complexed with selenium and mitigated the expected toxicity.

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Shelton, L.R., and L.K. Miller. 1988. Water-quality data, San Joaquin Valley California, March 1985 to March 1987. U.S. Geological Survey Open File Report 88-479. 210 pp.

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Hamilton, S.J., and R.H. Wiedmeyer. 1990. Bioconcentration of a mixture of boron, molybdenum, and selenium in chinook salmon. Transactions of the American Fisheries Society (In review).

In-house report

U.S. Fish and Wildlife Service Research Information Bulletin #89-47: Young salmon may be adversely affected by boron and selenium.

Table 1. Nominal and some measured (in parentheses) concentrations (ppb) of a mixture of inorganics used in a 120-day chronic toxicity exposure of young chinook salmon and reared in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water.

	Treatment (ppb)					
Chemical	0X	0.25X	0.5X	1X	2X	4X
Arsenate	0	0.03	0.06	0.12	0.24	0.48
Boron	0	325	650	1300	2600	5200
Cadmium	0	0.025	0.05	0.10	0.20	0.40
	(0)	(0.02)	(0.04)	(0.08)	(0.13)	(0.26)
Chromate	0	0.875	1.75	3.5	7	14
Copper	0	0.125	0.25	0.5	1	2
	(0.62)	(0.78)	(0.85)	(1.14)	(1.47)	(2.12)
Iron (III)	0	2.5	5	10	20	40
Lead	0	0.05	0.1	0.2	0.4	0.8
Manganese	0	0.5	1	2	4	8
Mercury	0	0.004	0.008	0.016	0.032	0.064
Molybdenum	0	1.875	3.75	7.5	15	30
Nickel	0	0.5	1	2	4	8
Selenate	0	7.5	15	30	60	120
Selenite	0	1.25	2.5	5	10	20
Total selenium	0	8.75	17.5	35	70	140
	(1.75)	(7.79)	(16.6)	(32)	(63)	(136)
Silver	0	0.025	0.05	0.1	0.2	0.4
Vanadium	0	0.75	1.5	3	6	12
Zinc	0	0.375	0.75	1.5	3	6

Table 2. Acute toxicity (LC50s in ppm: 95% confidence intervals in parentheses) of various inorganics to chinook salmon (0.5 g) in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water. Also presented are the concentrations of inorganics in standardized San Luis Drain water and the ratio of 96-hour LC50 value to San Luis Drain concentration for each inorganic.

Chemical	Exposure period (hour)				San Luis Drain conc. (ppm)	Ratio
	24		96			
Cadmium	>0.100		0.0259	(0.0196-0.0342)	0.001	26
Copper	0.145	(0.122-0.173)	0.058	(0.041-0.083)	0.005	12
Mercury	0.168	(0.130-0.216)	0.101	(0.078-0.130)	0.0002	505
Zinc	5.53	(4.37-7.00)	1.27	(1.02-1.59)	0.015	85
Vanadium	39.2	(32.8-46.7)	16.5	(14.0-19.5)	0.03	550
Arsenite	59.6	(50.3-70.7)	25.1	(19.3-32.7)	0.0012	21000
Arsenate						
Unbuffered	167	(130-216)	90.4	(70.2-116)	0.0012	75330
Buffered	>470		167	(120-233)	0.0012	139170
Chromate	261	(207-329)	111	(87-142)	0.035	3170
Boron	>1000		725	(590-890)	13	56
Molybdenum	>1000		>1000		0.75	>1333

Table 3. Concentrations (ppb) and the individual toxic units (in parentheses) of each element in two mixtures that produced 50% mortality in a 96-hour period to chinook salmon (0.9 g) tested in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water and the acute joint toxicities of each mixture.

Chemical	Mix I	Mix II
Mercury	0.85 (0.008)	0.89 (0.009)
Cadmium	5.3 (0.205)	5.6 (0.216)
Silver	5.3 (<0.001)	5.6 (<0.001)
Arsenate	6.4 (<0.001)	6.7 (<0.001)
Lead[a]	10.6	11.1
Copper	26.6 (0.488)	27.8 (0.510)
Zinc	7.8 (0.063)	83.4 (0.066)
Manganese[a]	106	111
Nickel[a]	106	111
Vanadium	160 (0.010)	167 (0.010)
Chromium	186 (0.002)	195 (0.002)
Molybdenum[b]	399 (<0.001)	417 (<0.001)
Boron[b]	69160 (0.095)	72280 (0.100)
Selenite	N.P.[c]	278 (0.019)
Selenate	N.P.	1670 (0.014)
Ratio (LC50: conncentration in SLD)	5.32	5.56
Joint Toxicity		
Toxic Units	0.871	0.946
Additive Index	0.148	0.057
(range)	(-0.639-0.165)	(-0.659-0.733)

[a] Toxic unit value not determined.

[b] Individual toxicities taken from Hamilton and Buhl (in prep.)

[c] Not present.

Table 4. Mean and standard error (in parentheses) of length (mm) and weight (g) of chinook salmon exposed to a mixture of 16 inorganics for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water. No significant differences from control at $P \leq 0.05$.

Days of exposure	Treatment					
	0X[a]	0.25X	0.5X	1X	2X	4X
Length						
30	45.6 (0.6)	45.4 (0.6)	45.1 (0.6)	45.7 (0.6)	44.7 (0.6)	47.0 (0.5)
60	57.1 (1.0)	57.8 (1.1)	55.0 (1.1)	58.1 (1.0)	54.9 (1.1)	59.8 (0.8)
90	72.9 (0.9)	70.4 (1.3)	70.7 (1.4)	72.2 (1.4)	70.6 (1.5)	73.3 (0.9)
120	84.8 (1.4)	84.0 (1.8)	81.7 (1.8)	83.1 (2.0)	82.1 (2.0)	85.0 (1.3)
Weight						
30	0.77 (0.03)	0.76 (0.04)	0.76 (0.04)	0.80 (0.03)	0.73 (0.04)	0.86 (0.03)
60	1.62 (0.08)	1.68 (0.09)	1.47 (0.09)	1.69 (0.08)	1.43 (0.09)	1.81 (0.07)
90	3.43 (0.13)	3.13 (0.17)	3.12 (0.17)	3.37 (0.18)	3.18 (0.19)	3.46 (0.14)
120	5.39 (0.29)	5.28 (0.32)	4.93 (0.33)	5.14 (0.34)	5.01 (0.36)	5.38 (0.26)

[a] Nominal and some measured concentrations of the 16-inorganic mixture are given in Table 1.

Table 5. Concentrations of cadmium, copper, selenium, and zinc (ppm dry weight) in whole-body of chinook salmon exposed to a mixture of 16 inorganics for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a fresh receiving water.

Days of exposure	Treatment					
	0X[a]	0.25X	0.5X	1X	2X	4X
Cadmium						
30	0.033	0.036	0.046	0.085	0.140	0.201
60	0.029	0.039	0.045	0.094	0.104	0.241
90	0.046	0.045	0.080	0.099	0.087	0.167
120	0.037	0.052	0.054	0.067	0.076	0.094
Copper						
30	3.35	3.60	3.58	4.07	3.89	3.38
60	3.07	3.14	3.25	3.53	3.12	3.66
90	2.68	2.50	2.65	2.61	2.53	2.34
120	3.14	2.86	3.26	2.77	2.74	3.04
Selenium						
30	2.04	2.13	2.37	2.68	3.25	4.29
60	1.38	1.69	1.97	2.11	2.56	4.07
90	1.27	1.36	1.37	1.61	1.69	2.19
120	1.05	1.13	1.19	1.26	1.17	0.72
Zinc						
30	132	126	129	126	124	121
60	121	145	154	142	156	157
90	120	127	124	130	126	129
120	124	150	121	107	120	109

[a] Nominal and some measured concentrations of the 16-inorganic mixture are given in Table 1.

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SAN LUIS DRAIN CONTAMINANTS IN BRACKISH WATER

Introduction

Agricultural wastewater from irrigation acitivites in the San Joaquin Valley of California has been reported to contain high concentrations of boron, chromium, iron, lead, molybdenum, vanadium, and zinc (Shelton and Miller, 1988). These elevated concentrations of inorganics were found in a number of drainage canals between Mendota and Gustine, including Kesterson Reservoir, and the San Luis Drain collector lines. Subsurface drainage water already enters fresh-water habitats of the Grasslands area, located south and adjacent to Kesterson, by way of large canals, drains, and small ditches. Entry of irrigation return flows contaminated with high concentrations of inorganics into the San Joaquin River and subsequent transport to the Sacramento-San Joaquin Delta could cause adverse effects on fish and wildlife. Likewise, completion of the San Luis Drain from Kesterson Reservoir to the Chipps Island area in the Delta, which is a drainwater management option, could directly deposit water laden with inorganics to aquatic habitats in the Delta.

Entry of elevated concentrations of inorganics into the Delta could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily in the presmolt life stages when fry are inhabiting the Delta prior to smoltification and seaward migration.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to inorganics in water qualities that simulate potential environmental conditions in brackish water. Acute toxicity studies were conducted with arsenate, arsenite, boron, cadmium, chromate, copper, mercury, vanadium, and zinc, tested individually, and with 13-inorganic and 15-inorganic mixtures; one chronic toxicity study was conducted with a 16-inorganic mixture.

Methods

Acute toxicity tests: The advanced fry life stage of fall-run chinook salmon was used in acute toxicity studies to determine the 96-hour LC50 values (i.e., concentration that kills 50% of the test animals in a 96-hour time period). The inorganics tested individually were arsenate, arsenite, boron, cadmium, chromate, copper, mercury, molybdenum, vanadium, and zinc. Arsenate toxicity was determined in a pH-buffered test and in a non-pH-buffered test because the form of arsenate used in toxicity tests (arsenic(V)oxide hydrate) caused a pH shift during the test, which ranged from pH 3.0 to 6.9 depending on the treatment. The pH of the buffered test was adjusted to pH 7.0 with a sodium hydroxide-sodium phosphate mixture. Tests with mixtures of inorganics included those given above (except arsenite) plus lead, manganese, nickel, selenate, selenite, and silver. Mixtures were formulated to simulate the environmental ratios in standardized San Luis Drain water.

Acute toxicity tests were conducted in accordance with accepted procedures from the U.S. Environmental Protection Agency and American Society for Testing and Materials. One deviation from those procedures in our studies was the use of standardized test waters that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 22.5-fold in a standardized brackish receiving water (about 1.2 ppthousand salinity). Actual drainage water was not used in toxicity studies because its water quality varies with location and time of year. The water quality of the test water, prior to addition of test chemicals, was pH 7.8, 343 ppm hardness, 37 ppm alkalinity as HCO_3, 39 ppm calcium, 60 ppm magnesium, 469 ppm sodium, 724 ppm chloride, and 288 ppm sulfate. This reconstituted test water was created by mixing high purity water from a wate softener-reverse osmosis-deionizer system with Instant Ocean and small amounts of various other salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. The LC50 values were calculated with the Litchfield-Wilcoxon method.

Chronic toxicity test: The advanced fry life stage of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to a waterborne mixture of 16 inorganics in the environmental ratios found in the San Luis Drain. The mixture of inorganics tested included arsenate, boron, cadmium, chromate, copper, ferric iron, lead, manganese, mercury, molybdenum, nickel, selenate, selenite, silver, vanadium, and zinc. The chronic toxicity test was conducted in a similar standardized test water as used in acute toxicity tests. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted water to each exposure aquarium at 15-minute intervals. Six duplicate nominal concentrations of the inorganic mixture were tested (Table 1). In each exposure aquarium throughout the study, mortality was recorded daily, fish behavior evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selected inorganic concentrations, and at 30-day intervals, fish samples were collected for measurement of selected inorganic concentrations, fish length and weight were measured, fish behavior was videotaped, and fish swimming performance was measured. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure.

Water samples from the chronic toxicity study were analyzed for concentrations of cadmium, copper, and selenium at the Yankton FRS, SD. Tissue samples from the chronic toxicity study were analyzed for concentrations of cadmium, copper, selenium, and zinc at NFCR-Columbia (MO).

Principal Findings

Acute toxicity tests: Toxicity to advanced chinook salmon of the inorganics tested varied considerably (Table 2) and is summarized in the following order of toxicity (selenium forms are included for comparison): mercury (most toxic) > cadmium = copper > > zinc > > selenite = vanadium > arsenite > arsenate (unbuffered) > selenate = chromate = arsenate buffered > > boron > molybdenum (least toxic). In these tests, four of the 10 inorganics had 96-hour LC50 values that were less than a factor of 100 of their concentrations in undiluted San Luis Drain water (boron, cadmium, copper, and mercury). In general, a factor of 100-fold difference between the 96-hour LC50 value and the expected environmental concentration is the minimal safety margin for protection of aquatic organisms against sublethal toxicity for most inorganics. Consequently, elevated concentrations of boron, cadmium, copper, and mercury in slightly diluted San Luis Drain water would cause toxic effects in aquatic organisms in a brackish receiving water.

The acute toxicity of a mixture of 13 inorganics (arsenate, boron, cadmium, chromate, copper, lead, manganese, mercury, molybdenum, nickel, silver, vanadium, and zinc) in their environmental ratios found in standardized San Luis Drain water was equal in its toxicity to juvenile chinook salmon tested in brackish water as when the 13-inorganic mixture contained selenate and selenite (Table 3). This result suggests that the waterborne toxicity of the mixture of inorganics was not dependent on the presence of selenate or selenite but rather on the joint toxicity of the other inorganic components of the mixture.

Based on an evaluation of toxic units (a toxic unit is the ratio of the LC50 concentration of an inorganic in a mixture to the LC50 concentration of an inorganic tested individually), the major toxic component of the inorganic mixture was copper (toxic unit 0.992-1.140) followed closely by boron, cadmium, and mercury (Table 3). Selenate and selenite had relatively low toxic unit values, thus indicating their small contribution to the waterborne toxicity of the mixture of inorganics that was tested in their environmental ratios found in standardized San Luis Drain water.

Both the 13-inorganic mixture with and without selenate and selenite showed antagonistic characteristics and, consequently, was less toxic to chinook salmon than expected based on the individual toxicity of the inorganic components of the mixture because the values for the additive toxicity index were negative (Table 3).

The hazard potential of the inorganic mixture with and without selenate and selenite to salmon populations was estimated by comparing the acute toxicity values with the expected environmental concentrations in the San Luis Drain. Based on the joint acute toxicity of the environmental mixture of inorganics, the calculated margin of safety for the mixture without selenium was 11.9, whereas for the mixture with selenium it was 13.7. Safety margins less than 100 indicate a high potential for adverse effects on aquatic resources, thus, the results of these acute toxicity tests indicate that the mixture of inorganics found in the San Luis Drain pose a "waterborne threat" to the health and well-being of juvenile chinook salmon in brackish water regardless of whether selenium is present in the mixture or not.

Chronic toxicity test: Preliminary results from the 120-day chronic toxicity study indicate that waterborne exposure to nominal concentrations of the mixture of 16 inorganics in the high concentration treatment (Table 1) for 120 days had no effect on survival or growth of chinook salmon (Table 4). Analysis of concentrations of cadmium, copper, selenium, and zinc in tissue samples revealed no significant accumulation of these elements in fish exposed to the mixture of 16 inorganics after 120 days of exposure (Table 5). There was no effect on survival of fish in the 10-day seawater challenge test. The high treatment concentration of the inorganic mixture tested in the chronic toxicity study was about 17 times lower than the 96-hour LC50 value for the mixture that included selenium in one acute toxicity test. Consequently, exposure of fish for 120 days to the high treatment should have caused significant mortality and it is unclear why it did not.

Work Remaining

The results of the chronic toxicity study with the advanced fry life stage of chinook salmon exposed to a mixture of 16 inorganics are awaiting further statistical analyses and analyses of fish behavior and swimming performance measurements.

Research Needs

Because no definitive tests were accomplished with molybdenum, future research should be conducted to determine its toxicity to two life stages of chinook salmon.

The lack of effects in the chronic toxicity test does not correspond with the results of the acute toxicity test with the 15-inorganic mixture and therefore the acute toxicity test should be repeated -- the only difference between the tests was the inclusion of ferric iron in the mixture used in the chronic toxicity test but not in the acute toxicity test. It is possible that the iron complexed with the selenium and mitigated the expected toxicity.

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Hamilton, S.J., and R.H. Wiedmeyer. 1990. Bioconcentration of a mixture of boron, molybdenum, and selenium to chinook salmon. Transactions of the American Fisheries Society (In review).

In-house report

U.S. Fish and Wildlife Service Research Information Bulletin #89-47: Young salmon may be adversely affected by boron and selenium.

Table 1. Nominal and some measured (in parentheses) concentrations (ppb) of a mixture of inorganics used in a 120-day chronic toxicity exposure of juvenile chinook salmon and reared in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water.

	Treatment (ppb)					
Chemical	0X	0.25X	0.5X	1X	2X	4X
Arsenate	0	0.06	0.12	0.24	0.48	0.96
Boron	0	650	1300	2600	5200	10400
Cadmium	0	0.05	0.10	0.20	0.40	0.80
	(0.03)	(0.08)	(0.14)	(0.22)	(0.42)	(0.80)
Chromate	0	1.75	3.5	7	14	28
Copper	0	0.25	0.5	1	2	4
	(2.0)	(2.5)	(2.8)	(3.6)	(4.2)	(6.2)
Iron (III)	0	5	10	20	40	80
Lead	0	0.1	0.2	0.4	0.8	1.6
Manganese	0	1	2	4	8	16
Mercury	0	0.008	0.016	0.032	0.064	0.128
Molybdenum	0	3.75	7.5	15	30	60
Nickel	0	1	2	4	8	16
Selenate	0	15	30	60	120	240
Selenite	0	2.5	5	10	20	40
Total selenium	0	17.5	35	70	140	280
	(0.02)	(13.2)	(30)	(64)	(123)	(270)
Silver	0	0.05	0.1	0.2	0.4	0.8
Vanadium	0	1.5	3	6	12	24
Zinc	0	0.75	1.5	3	6	12

Table 2. Acute toxicity (LC50s in ppm: 95% confidence intervals in parentheses) of various inorganics to chinook salmon (1.6-2.4 g) in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water. Also presented are the concentrations of inorganics in standardized San Luis Drain water and the ratio of 96-hour LC50 value to San Luis Drain concentration for each inorganic.

Chemical	Exposure period (hour)				San Luis Drain conc. (ppm)	Ratio
	24		96			
Mercury	0.019	(0.015-0.022)	0.017	(0.013-0.022)	0.0002	85
Cadmium	>0.130		0.057	(0.047-0.069)	0.001	57
Copper	0.078	(0.063-0.097)	0.060	(0.051-0.071)	0.005	12
Zinc	12.6	(10.0-17.0)	2.88	(1.97-4.21)	0.015	192
Vanadium	46.5	(39.7-54.5)	16.5	(13.9-19.6)	0.03	550
Arsenite	56.5	(46.0-69.3)	21.4	(18.1-25.3)	0.0012	17800
Arsenate						
Unbuffered	78.8	(60.0-100)	66.5	(55.4-79.8)	0.0012	55400
Buffered	265	(224-314)	157	(119-208)	0.0012	130800
Chromate	310	(242-398)	144	(121-172)	0.035	4100
Boron	>1000		600	(511-706)	13	46
Molybdenum	>1000		>1000		0.75	>1333

Table 3. Concentrations (ppb) and the individual toxic units (in parentheses) of each element in two mixtures that produced 50% mortality in a 96-hour period to chinook salmon (1.8 g) tested in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water and the acute joint toxicities of each mixture.

Chemical	Mix I	Mix II
Mercury	1.90 (0.113)	2.19 (0.130)
Cadmium	11.9 (0.208)	13.7 (0.240)
Silver	11.9 (<0.001)	13.7 (<0.001)
Arsenate	14.3 (<0.001)	16.4 (<0.001)
Lead [a]	23.8	27.4
Copper	59.5 (0.992)	68.4 (1.140)
Zinc	179 (0.062)	205 (0.071)
Manganese [a]	238	274
Nickel [a]	238	274
Vanadium	357 (0.022)	410 (0.025)
Chromium	417 (0.003)	479 (0.003)
Molybdenum [b]	893 (<0.001)	1026 (<0.001)
Boron [b]	154700 (0.258)	177840 (0.297)
Selenite	N.P.	684 (0.030)
Selenate	N.P.	4100 (0.023)
Ratio (LC50: concentration in SLD)	11.9	13.7
Joint Toxicity		
Toxic Units	1.658	1.959
Additive Index	-0.658	-0.959
(range)	(-1.584--0.065)	(-1.823--0.362)

[a] Toxic unit value not determined.

[b] Individual toxicities taken from Hamilton and Buhl (in prep.)

[c] Not present.

Table 4. Mean and standard error (in parentheses) of length (mm) and weight (g) of chinook salmon exposed to a mixture of 16 inorganics for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water. No significant differences from control at P≤0.05.

Days of exposure	Treatment					
	0X[a]	0.25X	0.5X	1X	2X	4X
Length						
30	106 (1)	105 (1)	103 (1)	104 (1)	104 (1)	104 (1)
60	119 (1)	117 (1)	115 (1)	116 (1)	118 (1)	117 (1)
90	134 (1)	131 (1)	130 (1)	131 (1)	133 (1)	132 (1)
120	149 (2)	145 (1)	144 (1)	145 (2)	147 (2)	146 (2)
Weight						
30	9.7 (0.3)	9.6 (0.3)	9.0 (0.3)	9.2 (0.3)	9.2 (0.3)	9.3 (0.3)
60	14.7 (0.6)	14.4 (0.5)	13.8 (0.5)	14.1 (0.5)	14.2 (0.5)	14.0 (0.5)
90	22.2 (0.8)	20.7 (0.6)	20.2 (0.7)	21.0 (0.8)	21.5 (0.8)	21.2 (0.7)
120	30.8 (1.1)	29.0 (0.8)	28.4 (1.0)	29.2 (1.1)	30.1 (1.1)	29.8 (1.1)

[a] Nominal and some measured concentrations of the 16-inorganic mixture are given in Table 1.

Table 5. Concentrations of cadmium, copper, selenium, and zinc (ppm dry weight) in whole-body of chinook salmon exposed to a mixture of 16 inorganics for 120 days in a reconstituted test water simulating dilution of the San Luis Drain in a brackish receiving water.

Days of exposure	Treatment					
	0X[a]	0.25X	0.5X	1X	2X	4X
Cadmium						
30	0.050	0.063	0.072	0.068	0.087	0.110
60	0.070	0.070	0.070	0.081	0.096	0.117
90	0.058	0.059	0.048	0.057	0.078	0.097
120	0.035	0.029	0.039	0.055	0.057	0.080
Copper						
30	3.86	3.56	3.43	2.55	3.68	3.28
60	3.96	3.25	3.71	3.03	4.05	3.01
90	3.08	2.51	3.25	3.05	2.06	2.26
120	2.51	3.50	4.39	3.26	3.30	3.07
Selenium						
30	1.04	1.09	1.16	1.12	1.31	1.50
60	1.06	1.19	1.13	1.23	1.25	1.43
90	1.04	0.95	1.02	1.06	1.03	1.24
120	1.02	0.99	1.03	1.13	0.87	1.16
Zinc						
30	157	148	157	147	136	158
60	141	136	133	148	142	150
90	142	143	136	127	130	104
120	134	123	128	131	107	138

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[a] Nominal and some measured concentrations of the 16-inorganic mixture are given in Table 1.

CHRONIC DIETARY EXPOSURE OF SALMON TO SELENIUM IN IN FRESHWATER AND BRACKISH WATER

Introduction

Following discovery of selenium contamination in the San Luis Drain and its terminus, Kesterson Reservoir, field investigations showed that selenium was being bioaccumulated in aquatic food-chain organisms at concentrations toxic to consumer animals. Elevated selenium concentrations were found in fish from the Helm Canal in the Grasslands, located south and adjacent to Kesterson (Saiki and May, 1988). Wetland habitats of the Grasslands receive subsurface drain water from large canals, drains, and small ditches. Elevated selenium concentrations were also found in fish from Mud Slough, which is the primary drainage system for the Grasslands; Mud Slough drains into the San Joaquin River (Saiki and May, 1988).

Discharge of water containing elevated concentrations of selenium in food-chain organisms to the San Joaquin River or to the Sacramento-San Joaquin Delta could potentially cause adverse impacts on salmonids from a dietary route of exposure. Toxicological impacts on salmonids would occur primarily in the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries. Additional impacts could occur on advanced fry inhabiting nursery areas in the Delta where they undergo parr-smolt transformation and migrate to the sea.

The present research was undertaken to determine the effects on chinook salmon of dietary exposure to selenium in water qualities that simulate potential environmental conditions in freshwater and brackish water.

Methods

Two chronic toxicity studies were conducted with chinook salmon exposed to selenium in the diet to determine the effects on survival, growth, and whole-body burdens of selenium. Fish were obtained as eyed eggs from the Merced River Fish Facility, Snelling, CA. In both studies three Oregon Moist Pellet diets were used; typically, 40% of the dry ingredients in the diet is salmon meal. In the test diets, half the salmon meal was replaced with fish meal made from ground, freeze-dried mosquitofish prior to mixing dry ingredients together and preparing the final product. The control diet contained meal made from uncontaminated mosquitofish collected from a reference site, one series of dietary selenium exposures contained meal made from mosquitofish collected from the San Luis Drain (termed SLD diet), and a second series of dietary selenium exposures contained meal made from uncontaminated mosquitofish that was spiked with selenomethionine (termed SEM diet).

In both toxicity studies, the concentrations of selenium tested in each of the two selenium-containing diets were 3.2, 5.3, 9.6, 18.2, and 35.4 ppm dry weight; the control diet contained 1.0 ppm selenium dry weight. Each dietary treatment was fed to 100 fish in two replicate aquaria. Both chronic toxicity tests were conducted in an intermittent-flow diluter which delivered 1 liter of reconstituted test water to each exposure aquarium at 15-minute intervals. In each exposure aquarium throughout both studies, mortality was recorded daily, fish length and weight was measured at 30-day intervals, and fish were sampled at 30-day intervals and water at 15-day intervals for selenium determinations.

The first study was conducted for 90 days and started with the swimup life stage and a reconstituted test water that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 37-fold in a standardized fresh receiving water. Actual drain water was not used in toxicity studies because its water quality varies with location and time of year. The water quality of the test water was pH 7.7, 210 ppm hardness, 109 ppm alkalinity as HCO_3, 45 ppm calcium, 23 ppm magnesium, 70 ppm sodium, 59 ppm chloride, and 186 ppm sulfate. This reconstituted test water was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. This study is termed the fresh-water study.

The second study was conducted for 120 days and started with advanced fry and a reconstituted test water that simulated a 22.5-fold dilution of standardized San Luis Drain water in a standardized brackish receiving water. The water quality of the test water was pH 7.4, 235 ppm sulfate, and 0.6 ppthousand salinity for the first 30 days while for the remainder of the study it was pH 6.9, 291 ppm sulfate, and 1.2 ppthousand salinity. This reconstituted test water was created as in the first study except that the majority of salts added were Instant Ocean sea salts. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 30 fish from each treatment following the 120-day exposure. This study is termed the brackish-water study.

Water samples from both chronic toxicity studies were analyzed for selenium concentrations at the Yankton FRS, SD. Tissue samples from both chronic toxicity studies were analyzed for selenium concentrations at NFCR-Columbia (MO).

Principal Findings

Analysis of concentrations of 22 elements in the diets revealed that the major toxic component in the SLD diet was selenium but concentrations of boron, chromium, and strontium were also elevated compared to concentrations in the control and SEM diets

(Table 1). Concentrations of selenium in water were below the limit of quantitation (0.59-1.45 ppb) in all dietary selenium exposures in both chronic toxicity studies. Selenium was not directly added to water in these experiments but was indirectly added to water from excretory products from fish and dissolution of diet; however, these sources of selenium were minimized by daily cleaning and siphoning out of feces and uneaten food from exposure aquaria.

Fresh-water study: Survival was reduced by exposure to 9.6 ppm selenium for 90 days in both dietary exposures (Table 2). Growth, as measured by length and weight, was reduced by exposure to ≥ 5.3 ppm selenium for 90 days in both dietary exposures (Table 3). Whole-body burdens of selenium were increased in fish fed ≥ 3.2 ppm selenium in both selenium-containing diets at all sampling periods (Table 4). A strong dose-response relation between concentration of dietary selenium and survival, growth, and whole-body burdens of selenium was apparent after 90 days of exposure to both selenium diets.

Brackish-water study: Survival was not affected in fish fed either selenium-containing diet for 120 days. In the SLD diet study, fish length was reduced significantly by exposure to ≥ 18.2 ppm selenium for 120 days whereas fish weight was reduced significantly by exposure to ≥ 9.6 ppm selenium (Table 5). In the SEM diet study, fish length and weight were both reduced significantly by exposure to 35.4 ppm selenium for 120 days (Table 5). Survival of fish in a 10-day seawater challenge test following 120 days of dietary exposure was reduced in fish fed 35.4 ppm selenium in both selenium diets (Table 6). Whole-body burdens of selenium were increased in fish fed ≥ 3.2 ppm selenium in both selenium-containing diets at all sampling periods (Table 7). A strong dose-response relation between the concentration of dietary selenium and growth was apparent after 120 days of exposure to the SLD diet but not to the SEM diet. Whole-body burdens of selenium in both studies were also correlated with exposure concentration of dietary selenium.

General Conclusions

In both studies, fish fed the SLD diets had slightly higher whole-body residues of selenium than those fed the SEM diets. This difference in selenium uptake between diet types may have been due to the presence of organic forms of selenium other than selenomethionine in the SLD diet, enhanced uptake protein-bound organoselenium in the SLD diet (compared to free selenomethionine in the SEM diet), or the presence of elevated concentrations of boron, chromium, and strontium in the SLD diet.

Whole-body concentrations of selenium in chinook salmon in both studies increased in a concentration-dependent manner, but fish did not bioaccumulate selenium to concentrations greater than those present in the food. Based on the results of these studies, if selenium concentrations exceed 3 to 5 ppm dry weight in food organisms in the San Joaquin River or the Sacramento-San Joaquin Delta, young chinook salmon would be adversely affected.

Research Needs

Although selenomethionine (i.e., SEM diet) was shown to be a good model for organic forms of selenium in the SLD diet in our study, there is little information on the specific organic forms of selenium in animal tissue. Consequently, research should be conducted to determine the organo-selenium forms in animal tissue.

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In-house reports

U.S. Fish and Wildlife Service Research Information Bulletin #88-35: Young salmon are adversely affected by dietary selenium.

U.S. Fish and Wildlife Service Research Information Bulletin #89-65: Body burdens of selenium linked to reduced growth and survival in chinook salmon.

Table 1. Mean and standard error (in parentheses) of elemental content (ppm diet dry weight) of experimental diets.

Element[a]	Diet type		
	Control	SLD	SEM
Al	1802 (28)	1847 (14)	1804 (7)
B	5.8 (2.8)	13.8 (2.8)	10.0 (5.3)
Ba	30.3 (0.6)	24.2 (0.7)	26.6 (1.4)
Be	0.05 (0.01)	0.03 (0.01)	0.04 (0)
Cr	2.8 (0)	5.7 (0.1)	2.8 (0)
Cu	13 (0)	18 (1)	18 (0)
Fe	761 (11)	899 (6)	776 (4)
Mg	1753 (7)	1833 (0)	1706 (21)
Mn	139 (0)	134 (2)	163 (0)
Ni	<1	3 (0)	<3
Se	1.0 (0)	35.4 (0.7)	35.5 (0.7)
Sr	49.0 (1.5)	83.7 (0)	48.9 (0.8)
V	0.7 (0.1)	0.7 (0.1)	0.8 (0)
Zn	195(1)	179 (0)	223 (0)

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[a] Elements not detected and their detection limit in ppm (in parentheses) were Ag (0.3), As (3), Cd (0.3), Mo (0.8), Pb (3), Sb (6), Sn (1), and Tl (10).

Table 2. Mean and standard error (in parentheses) of percent survival of chinook salmon exposed to selenium in the diet and reared in a reconstituted fresh water.

Diet type	Dietary selenium concentration (ppm)[a]	Exposure period (day)		
		30	60	90
Control	1.0	99.0(1.0)	99.0(1.0)	66.7(4.1)
SLD	3.2	99.0(1.0)	97.3(1.6)	56.0(11.0)
	5.3	98.0(2.0)	93.0(2.7)	44.7(10.4)
	9.6	100.0(0)	95.0(1.7)	31.2(15.7)**
	18.2	99.0(1.0)	92.4(4.8)	20.9(11.7)**
	35.4	99.0(1.0)	89.0(3.1)*[b]	18.2(11.0)**
SEM	3.2	100.0(0)	100.0(0)	72.5(12.5)
	5.3	100.0(0)	95.0(1.7)	45.0(10.2)
	9.6	99.0(1.0)	94.1(4.0)	37.5(13.1)*
	18.2	99.0(1.0)	92.4(4.8)	2.4(11.3)**
	35.4	84.0(9.8)	62.5(14.0)**	10.7(6.8)**

[a] Diet dry weight.

[b] Asterisks indicate significant difference from the control (ANOVA: *$P \leq 0.10$ or **$P \leq 0.05$).

Table 3. Mean and standard error (in parentheses) of total length (mm) and weight (g) of chinook salmon exposed to selenium in the diet and reared in a reconstituted fresh water.

Diet type	Dietary selenium concentration (ppm)[a]	Length			Weight		
		30[b]	60	90	30	60	90
Control	1.0	51.1(0.5)	69.9(0.8)	82.4(1.0)	1.25(0.04)	3.35(0.12)	5.30(0.19)
SLD	3.2	48.5(0.5)**[c]	64.9(0.8)**	80.8(1.3)	1.08(0.03)**	2.68(0.11)**	5.01(0.27)
	5.3	49.0(0.6)**	66.0(0.9)	77.8(1.1)**	1.08(0.04)**	2.76(0.11)**	4.50(0.21)*
	9.6	48.7(0.5)**	65.8(1.0)**	79.1(1.6)	1.07(0.03)**	2.80(0.14)**	4.40(0.28)*
	18.2	48.9(0.5)**	64.5(1.0)**	74.7(1.6)**	1.09(0.03)**	2.62(0.12)**	3.77(0.30)**
	35.4	45.2(0.4)**	54.2(0.8)**	58.0(1.2)**	0.85(0.03)**	1.40(0.06)**	1.58(0.09)**
SEM	3.2	49.5(0.6)	67.8(1.0)	81.0(1.6)	1.16(0.04)	3.08(0.14)	5.13(0.32)
	5.3	49.9(0.5)	68.6(1.0)	82.9(1.6)	1.17(0.03)	3.22(0.15)	5.38(0.32)
	9.6	50.0(0.5)	67.6(1.0)	79.3(2.0)	1.18(0.03)	3.07(0.13)	4.71(0.41)
	18.2	48.2(0.5)*	64.4(1.0)**	71.7(2.5)**	1.05(0.03)*	2.61(0.12)**	3.26(0.28)**
	35.4	43.8(0.5)**	51.9(1.2)**	49.0(2.5)**	0.75(0.03)**	1.25(0.09)**	0.98(0.13)**

[a] Diet dry weight.
[b] Days of exposure.
[c] Asterisks indicate significant differences from the control (ANOVA; *P≤0.10 or **P≤0.05).

Table 4. Mean and standard error (in parentheses) of whole-body concentrations (ppm dry weight) of selenium in chinook salmon exposed to selenium in the diet and in a reconstituted fresh water.

Diet type	Dietary selenium concentration (ppm)[a]	Exposure period (day)		
		30	60	90
Control	1.0	1.0 (0)	0.9 (0)	0.8 (0)
SLD	3.2	3.4 (0.2)**[b]	3.3 (0.1)**	2.7 (0.2)**
	5.3	3.9 (0.2)**	4.5 (0.1)**	4.0 (0.1)**
	9.6	6.8 (0.2)**	8.4 (0)**	6.5**
	18.2	10.6 (0.3)**	13.3 (0.5)**	14.3 (0.5)**
	35.4	21.1 (0.4)**	29.4 (0.2)**	33.4**
SEM	3.2	2.4 (0)**	2.0 (0)**	1.7 (0.2)**
	5.3	3.1 (0)**	3.1 (0.2)**	2.6 (0.1)**
	9.6	5.1 (0.3)**	5.3 (0.1)**	5.4 (0.3)**
	18.2	8.5 (0.4)**	10.4 (0.1)**	10.8 (0.9)**
	35.4	16.0 (1.8)**	23.4 (0.8)**	27.8 (6.6)**

[a] Diet dry weight.

[b] Asterisks indicate significant difference from the control (ANOVA: $^{*}P \leq 0.10$ or $^{**}P \leq 0.05$).

Table 5. Mean and standard error (in parentheses) of total length (mm) and weight (g) of chinook salmon exposed to selenium in the diet and reared in a reconstituted brackish water.

Diet type	Dietary selenium concentration (ppm)[a]	Length				Weight			
		30[b]	60	90	120	30	60	90	120
Control	1.0	84.7 (1.2)	98.5 (2.3)	109.4 (3.3)	126.4 (4.3)	5.94 (0.28)	10.17 (0.76)	14.71 (1.38)	22.10 (2.29)
SLD	3.2	85.7 (1.0)	100.9 (1.7)	112.8 (2.5)	128.5 (3.9)	6.04 (0.21)	10.69 (0.54)	15.77 (1.03)	23.41 (1.94)
	5.3	83.9 (1.2)	96.7 (2.1)	107.2 (3.1)	123.4 (4.0)	5.73 (0.25)	9.47 (0.64)	13.62 (1.16)	20.19 (1.85)
	9.6	85.3 (0.9)	97.0 (1.7)	107.7 (2.6)	119.1 (3.4)	5.96 (0.20)	9.44 (0.51)	13.16 (0.98)	17.68 (1.54)**
	18.2	84.1 (1.1)	95.0 (2.0)	104.3 (2.8)	114.5 (4.3)**	5.78 (0.23)	9.03 (0.58)	12.68 (1.07)*	16.84 (1.99)**
	35.4	81.4 (0.9)*[c]	95.4 (1.6)	101.3 (2.1)**	110.5 (2.7)**	5.17 (0.20)	8.55 (0.44)**	10.30 (0.72)**	13.04 (0.94)**
SEM	3.2	86.4 (1.1)	99.7 (1.7)	110.3 (2.4)	124.2 (3.5)	6.28 (0.23)	10.24 (0.52)	14.76 (0.90)	20.53 (1.79)
	5.3	85.5 (1.2)	98.9 (2.1)	109.4 (3.1)	121.9 (4.2)	6.18 (0.24)	10.42 (0.69)	14.65 (1.25)	20.07 (2.06)
	9.6	84.6 (1.2)	96.9 (2.3)	105.7 (3.3)	115.7 (5.0)	5.78 (0.27)	9.60 (0.75)	13.30 (1.35)	18.29 (2.55)
	18.2	87.0 (1.1)	102.6 (1.8)	115.3 (2.5)	134.5 (2.7)	6.48 (0.24)	11.26 (0.60)	16.49 (1.04)	24.87 (1.50)
	35.4	81.6 (1.2)	93.9 (1.9)	97.5 (2.6)**	108.0 (4.0)**	5.32 (0.23)	8.59 (0.54)	9.57 (0.84)**	12.93 (1.57)**

[a] Diet dry weight.
[b] Days of exposure.
[c] Asterisks indicate significant differences from the control (ANOVA; $*P \leq 0.10$ or $**P \leq 0.05$).

Table 6. Mean and standard error (in parentheses) of percent survival of chinook salmon exposed to a 10-day seawater (28 ppthousand salinity) challenge test following exposure to selenium in the diet for 120 day and reared in a reconstituted brackish water.

Diet type	Dietary selenium concentration (ppm)[a]	% survival
Control	1.0	87(7)
SLD	3.2	67(7)
	5.3	93(7)
	9.6	73(7)
	18.2	73(7)
	35.4	37(10)**[b]
SEM	3.2	97(3)
	5.3	77(3)
	9.6	63(3)
	18.2	60(20)
	35.4	24(9)**

[a] Diet dry weight.

[b] Asterisks indicate significant difference from the control (ANOVA: *$P \leq 0.10$ or **$P \leq 0.05$).

Table 7. Mean and standard error (in parentheses) of whole-body concentrations (ppm dry weight) of selenium in chinook salmon exposed to selenium in the diet and in a reconstituted brackish water.

Diet type	Dietary selenium concentration (ppm)[a]	Exposure period (day)			
		30	60	90	120
Control	1.0	1.0(0)	0.8(0)	0.7(0.1)	0.8(0)
SLD	3.2	2.4(0)**[b]	2.1(0.1)**	2.6(0.2)**	2.7(0.1)**
	5.3	3.5(0.2)**	3.7(0)**	4.1(0.6)**	3.6(0.2)**
	9.6	6.0(0)**	6.8(0.1)**	7.7(0.3)**	8.0(0.4)**
	18.2	10.0(0.1)**	11.8(0.2)**	14.1(0.8)**	14.4(0.7)**
	35.4	19.7(1.3)**	22.6(0.5)**	29.4(0.5)**	28.8(1.6)**
SEM	3.2	1.9(0.2)**	1.8(0)**	2.0(0.1)**	1.8(0.1)**
	5.3	2.5(0.3)**	2.9(0.3)**	3.4(0)**	3.5(0.3)**
	9.6	4.6(0)**	5.6(0)**	6.4(0)**	6.0(0.7)**
	18.2	8.6(0.4)**	10.5(0.2)**	10.8(0.6)**	12.6(0.2)**
	35.4	15.2(0.7)**	19.3(1.0)**	24.0(0.1)**	23.2(0.6)**

[a] Diet dry weight.

[b] Asterisks indicate significant difference from the control (ANOVA: *$P \le 0.10$ or **$P \le 0.05$).

CHRONIC DIETARY AND WATERBORNE EXPOSURE OF SALMON TO SELENIUM IN FRESHWATER AND BRACKISH WATER

Introduction

Field investigations in areas surrounding Kesterson Reservoir, the terminus of the San Luis Drain, have shown that elevated concentrations of selenium are present in both water and food-chain organisms (Saiki and May, 1988). The source of the selenium is from large canals, drains, and small ditches that deposit subsurface drainage water in the Grasslands, located south and adjacent to Kesterson Reservoir. The primary drainage system for the Grasslands is Mud (north) and Salt sloughs, which are tributaries of the San Joaquin River. Entry of water and food-organisms containing elevated concentrations of selenium into the San Joaquin River and subsequent transport to the Sacramento-San Joaquin Delta could cause adverse effects on fish and wildlife. Likewise, completion of the San Luis Drain from Kesterson Reservoir to the Chipps Island area in the Delta, which is a drainwater management option, could directly deliver selenium-laden water and food-chain organisms to aquatic habitats in the Delta. The presence of selenium in both water and food-organisms allows two routes of uptake that could affect fish and wildlife resources.

Salmonids in the San Joaquin River or the Delta could be exposed to selenium in water and food-organisms if entry of selenium-laden water to these two aquatic habitats is allowed to occur. Toxicological impacts on salmonids would occur primarily in the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries. Additional impacts could occur on advanced fry inhabiting nursery areas in the Delta where they undergo parr-smolt transformation and migrate to the sea.

The present research was undertaken to determine the effects on chinook salmon of combined waterborne and dietary exposure to selenium in water qualities that simulate potential environmental conditions in freshwater and brackish water.

Methods

Two chronic toxicity studies were conducted with chinook salmon exposed to selenium in both water and diet to determine the effects on survival, growth, and whole-body burdens of selenium. Fish were obtained as eyed eggs from Iron Gate Salmon and Steelhead Hatchery, Hornbrook, CA.

In the first study, the swimup life stage was exposed for 120 days to 12 duplicated treatments using an intermittent-flow proportional diluter which delivered 1 liter of reconstituted test water to each exposure aquarium at 15-minute intervals. Nominal concentrations of selenium that comprised the treatments were 0, 17, and 70 ppb (6:1 ratio of selenate:selenite) in water and 0, 4, 9, and 18 ppm (dry weight) in the form of selenomethionine in the diet.

The matrix at the right shows the 12 treatment combinations that were tested in a reconstituted test water that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 37-fold in a standardized fresh receiving water. Actual drain water was not used in toxicity studies because its water quality consistency varies due to location and time of year. In each exposure aquarium throughout the study, mortality was recorded daily, fish behavior evaluated weekly, water samples collected weekly for measurement of

		Water		
		0	17	70
D	0	1	5	9
i	4	2	6	10
e	9	3	7	11
t	18	4	8	12

eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and at 30-day intervals, fish samples were collected for measurement of selenium concentrations, fish length and weight were measured, fish behavior was videotaped, and fish swimming performance was measured. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 20 fish from each treatment following the 120-day exposure. The water quality of the exposure water was pH 7.6, 211 ppm hardness, 110 ppm alkalinity as HCO_3, 46 ppm calcium, 24 ppm magnesium, 70 ppm sodium, 60 ppm chloride, and 186 ppm sulfate. This reconstituted test water was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. This study is termed the fresh-water study.

In the second study, advanced fry were exposed for 120 days to 12 duplicated treatments as in the first study. Nominal concentrations of selenium that comprised the treatments were 0, 35, and 140 ppb (6:1 ratio of selenate:selenite) in water and 0, 9, 18, and 36 ppm (dry weight) in the form of selenomethionine in the diet.

The matrix at the right shows the 12 treatment combinations that were tested in a reconstituted test water that simulated a 22.5-fold dilution of standardized San Luis Drain water in a standardized brackish receiving water. The water quality of the exposure water was pH 7.4, 246 ppm sulfate, and 0.6 ppthousand salinity for the first 30 days while for the remainder of the study it was pH 7.0, 287 ppm sulfate, and 1.2 ppthousand salinity. This reconstituted test water was created as in the first study except that the majority of salts added were Instant

		Water		
		0	35	140
D	0	1	5	9
i	9	2	6	10
e	18	3	7	11
t	36	4	8	12

Ocean sea salts. In each exposure aquarium throughout the study, mortality was recorded daily, fish behavior evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and at 30-day intervals, fish samples were collected for measurement of selenium concentrations, fish length and weight were measured, and fish behavior was videotaped. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure. This study is termed the brackish-water study.

Water samples from both chronic toxicity studies were analyzed for selenium concentrations at the Yankton FRS, SD. Tissue samples from both chronic toxicity studies were analyzed for selenium concentrations at NFCR-Columbia (MO).

Principal Findings

Fresh-water study: The control and selenium-laden diets were analyzed for concentrations of boron, cadmium, selenium, and zinc. The control and treatment diets contained similar concentrations of boron (2.2-3.2 ppm), cadmium (0.04 ppm), and zinc (175-185 ppm); however, the control diet contained 0.87 ppm selenium (dry weight) and the selenium diets contained 5.0, 9.2, and 17.4 ppm selenium.

The measured waterborne concentrations of selenium in unfiltered water samples were 0 (control), 13, and 56 ppb compared with nominal concentrations of 0, 17, and 70 ppb, respectively. Concentrations of selenium in unfiltered aquaria exposure water were nearly identical with concentrations of selenium measured in water samples filtered through a glass fiber filter and then a 0.4 μm polycarbonate filter. The measured waterborne concentrations of selenium in filtered water samples were 0 (control), 14, and 62 ppb.

There was no effect on survival of chinook salmon exposed to waterborne and dietary selenium for 120 days. Fish length and weight appeared to be reduced in fish exposed to the highest waterborne and dietary treatment after 120 days (Table 2).

Whole-body burdens of selenium in chinook salmon in the fresh-water study seemed to be highly influenced by dietary selenium exposure and only slightly influenced by waterborne selenium exposure (Table 2). At each exposure sample period and within a specific dietary selenium exposure, whole-body burdens of selenium were only slightly increased with increasing waterborne selenium exposure concentrations. However, at each exposure sample period and within a specific waterborne selenium exposure concentration, whole-body burdens of selenium were greatly increased with increasing dietary selenium exposure. Whole-body burdens of selenium in chinook salmon seemed to be due almost exclusively to dietary exposure to selenium, but selenium was not accumulated in fish to concentrations greater than those in the test diets.

The high whole-body concentrations of selenium in fish fed the 9.2 and 17.4 ppm diets after 30, 60, 90, and 120 days of exposure should have caused increased mortality and reduced growth based on the results of other studies with dietary selenium fed to young chinook salmon. It is unclear why fish with high whole-body concentrations of selenium did not exhibit adverse effects.

Brackish-water study: The selenium concentrations in the control and selenium-laden diets were 0.87 (control), 9.2, 17.4, and 35.4 ppm (dry weight). The measured waterborne concentrations of selenium were 0.1 (control), 32, and 112 ppb compared with nominal concentrations of 0, 35, and 140 ppb, respectively.

After 10 days of exposure, there was a large number of dead fish in all exposure aquaria and in one replicate because the compressed air supplying supplemental oxygen to the exposure aquaria was inadvertently left off overnight. Consequently, the study was continued on but with only one replicate of treatments with the realization that no statistical analyses could be accomplished on the data because of the lack of replicate treatments. No toxicant-induced mortality or effects on fish growth (Table 3) seemed to occur during the study.

Whole-body burdens of selenium in chinook salmon in the brackish-water study followed a trend nearly identical to that observed in the fresh-water study (Table 4). Tissue residues seemed to be due almost exclusively to dietary exposure to selenium. In fish fed ≥ 9.2 ppm selenium in the diet, whole-body concentrations in fish were only about 30% of the concentrations in test diets at each of the sampling periods. This low level of selenium accumulation in advanced fry suggests some kind of regulatory mechanism for selenium that is not present in young fry because in the fresh-water study, young fry accumulated about 80% of the selenium in the test diet. In other studies where chinook salmon were exposed to either waterborne or dietary selenium, advanced fry typically accumulated less selenium than young fry.

Work Remaining

The results of the fresh-water study are awaiting further statistical analyses and analyses of fish behavior and swimming performance measurements.

Research Needs

The lack of effects in the chronic toxicity test do not correspond with the results of other research with chinook salmon or trout exposed to selenium and therefore the combined waterborne and dietary selenium study should be repeated.

Literature Cited

Saiki, M.K., and T.W. May. 1988. Trace elements in bluegills and common carp from the lower San Joaquin River, California, and its tributaries. Sci. Total Environ. 74: 199-217.

Literature Generated From These Studies

Hamilton, S.J., R.H. Wiedmeyer, and F.A. Bullard. Chronic toxicity of combined water and dietary selenium to chinook salmon (In preparation -- intended outlet is Archives of Environmental Contamination and Toxicology).

Hamilton, S.J., R.H. Wiedmeyer, and F.A. Bullard. Chronic toxicity of waterborne selenium and other inorganics to chinook salmon (In preparation -- intended outlet is Archives of Environmental Contamination and Toxicology).

Table 1. Mean and standard error (in parenthesis) of length (mm) and weight (g) of chinook salmon exposed to selenium in the water and diet for 120 days and reared in a water quality simulating dilution of standardized San Luis Drain water in a fresh receiving water.

Days of exposure		Waterborne selenium (ppb)	Dietary selenium (ppm dry weight)			
			0.9	5.0	9.2	17.4
30						
	Length	0	49.8 (0.3)	49.1 (0.2)	50.5 (0.3)	49.7 (0.3)
		13	49.9 (0.3)	49.8 (0.3)	49.4 (0.3)	49.8 (0.3)
		56	49.4 (0.3)	50.0 (0.3)	49.3 (0.3)	49.9 (0.3)
	Weight	0	1.06 (0.02)	1.03 (0.02)	1.11 (0.02)	1.06 (0.02)
		13	1.08 (0.02)	1.08 (0.02)	1.02 (0.02)	1.07 (0.02)
		56	1.03 (0.02)	1.07 (0.02)	1.04 (0.02)	1.08 (0.02)
60						
	Length	0	61.9 (0.5)	61.8 (0.4)	63.2 (0.5)	62.1 (0.6)
		13	61.9 (0.5)	63.1 (0.6)	62.3 (0.6)	63.0 (0.6)
		56	63.2 (0.6)	63.4 (0.6)	61.8 (0.6)	60.7 (0.5)
	Weight	0	2.10 (0.06)	2.12 (0.05)	2.27 (0.06)	2.18 (0.07)
		13	2.11 (0.05)	2.25 (0.07)	2.17 (0.07)	2.29 (0.07)
		56	2.30 (0.07)	2.31 (0.08)	2.14 (0.07)	2.00 (0.06)

Table 1. (continued)

Days of exposure		Waterborne selenium (ppb)	Dietary selenium (ppm dry weight)			
			0.9	5.0	9.2	17.4
90	Length	0	73.9 (0.8)	73.9 (0.7)	75.6 (1.0)	74.6 (1.1)
		13	74.0 (0.8)	74.8 (0.8)	73.9 (0.8)	76.5 (0.8)
		56	76.2 (1.1)	75.8 (1.1)	74.4 (0.9)	71.3 (0.8)
	Weight	0	3.63 (0.13)	3.80 (0.11)	4.04 (0.17)	3.96 (0.19)
		13	3.74 (0.13)	3.84 (0.13)	3.64 (0.12)	4.20 (0.15)
		56	4.15 (0.19)	4.09 (0.19)	3.88 (0.17)	3.33 (0.13)
120	Length	0	86.4 (1.1)	85.9 (1.2)	88.0 (1.9)	87.2 (1.6)
		13	86.0 (1.1)	84.9 (1.2)	84.5 (1.0)	88.8 (1.3)
		56	89.5 (1.9)	86.9 (1.5)	86.4 (1.6)	81.8 (1.1)
	Weight	0	5.79 (0.25)	5.94 (0.27)	6.62 (0.46)	6.09 (0.42)
		13	6.00 (0.23)	5.60 (0.28)	5.55 (0.20)	6.63 (0.34)
		56	6.88 (0.46)	6.20 (0.34)	6.17 (0.41)	5.06 (0.22)

Table 2. Mean and standard error (in parentheses) of whole-body concentrations of selenium (ppm dry weight) in chinook salmon exposed to selenium in water and diet for 120 days and reared in a water quality simulating dilution of standardized San Luis Drain water in a fresh receiving water.

Days of exposure	Waterborne selenium (ppb)	Dietary selenium (ppm dry weight)			
		0.9	5.0	9.2	17.4
30	0	1.26 (0.04)	3.13 (0.16)	5.75 (0.01)	9.20 (0.48)
	13	1.42 (0.14)	3.43 (0.32)	5.06 (0.04)	8.30 (0.37)
	56	1.67 (0.03)	3.66 (0.01)	5.57 (0.17)	9.11 (0.83)
60	0	1.17 (0.05)	3.62 (0.05)	5.77 (0.09)	11.65 (0.45)
	13	1.51 (0.12)	5.11 (0.74)	5.72 (0.16)	11.35 (0.75)
	56	2.11 (0.41)	4.06 (0.69)	6.69 (0.18)	12.30 (0.80)
90	0	1.14 (0.11)	3.33 (0.02)	5.53 (0.25)	10.02 (0.58)
	13	1.18 (0)	3.67 (0.30)	4.68 (1.66)	12.50 (0.80)
	56	1.64 (0.18)	3.79 (0.29)	5.04 (0.20)	9.09 (3.01)
120	0	0.94 (0.01)	3.06 (0.18)	5.41 (0.16)	11.45 (0.95)
	13	1.02 (0.02)	3.50 (0.36)	5.44 (0.26)	11.35 (0.05)
	56	1.29 (0.13)	3.42 (0.45)	6.22 (0.50)	11.85 (0.95)

Table 3. Mean length (mm) and weight (g) of chinook salmon exposed to selenium in the water and diet for 120 days and reared in a water quality simulating dilution of standardized San Luis Drain water in a brackish receiving water. Data are from one replicate of treatments: the other replicate was lost after 10 days of exposure.

Days of exposure		Waterborne selenium (ppb)	Dietary selenium (ppm dry weight)			
			0.9	9.2	17.4	35.4
30						
	Length	0	100	104	104	97
		32	107	106	103	104
		112	107	104	104	101
	Weight	0	9.8	11.1	10.8	8.8
		32	11.9	11.3	10.5	10.7
		112	12.1	10.9	10.8	9.8
60						
	Length	0	114	117	115	106
		32	120	119	116	116
		112	121	115	116	110
	Weight	0	15.4	16.5	15.1	12.0
		32	17.7	16.4	15.6	15.2
		112	17.5	15.4	15.8	13.2
90						
	Length	0	124	127	128	118
		32	131	130	130	127
		112	131	126	128	120
	Weight	0	18.7	21.5	20.9	17.7
		32	22.3	21.5	21.2	20.8
		112	22.2	20.2	21.8	18.3

Table 3. (continued)

Days of exposure		Waterborne selenium (ppb)	Dietary selenium (ppm dry weight)			
			0.9	9.2	17.4	35.4
120						
	Length	0	133	134	137	127
		32	141	139	139	134
		112	142	137	136	130
	Weight	0	24.1	25.9	26.9	21.6
		32	29.5	27.2	26.3	23.6
		112	28.9	26.2	26.2	23.0

Table 4. Mean of whole-body concentrations of selenium (ppm dry weight)in chinook salmon exposed to selenium in water and diet for 120 days and reared in a water quality simulating dilution of standardized San Luis Drain water in a brackish receiving water. Data are from one replicate of treatments: the other replicate was lost after 10 days of exposure.

Days of exposure	Waterborne selenium (ppb)	Dietary selenium (ppm dry weight)			
		0.9	9.2	17.4	35.4
30	0	0.78	2.29	4.38	7.35
	32	0.92	2.53	4.53	7.26
	112	0.99	2.93	4.44	7.70
60	0	0.85	3.01	4.78	9.00
	32	0.82	2.67	4.51	6.53
	112	0.97	3.60	5.96	9.10
90	0	0.60	3.45	7.70	11.8
	32	0.70	3.74	6.36	12.8
	112	1.08	3.60	6.19	12.9
120	0	0.77	3.56	6.13	13.9
	32	0.77	3.40	6.29	14.0
	112	1.13	4.32	6.35	13.2

TOLERANCE OF JUVENILE FALL CHINOOK SALMON TO SELENIUM EXPOSURE FROM WATER AND THE FOOD CHAIN: IMPACTS ON SMOLTIFICATION AND EARLY MARINE SURVIVAL

Introduction

The chinook salmon (*Oncorhynchus tshawytscha*) runs native to the Central Valley of California are a valuable renewable fishery resource that could be adversely affected if selenium (Se) from irrigation return flows contaminated surface waters such as the Sacramento River or the San Joaquin River. In order to evaluate disposal alternatives for San Joaquin Valley drainwater, we have determined safe exposure levels (Maximum Acceptable Toxicant Concentrations - MATC's) for fall chinook salmon smolts to organoselenium in the diet and to inorganic Se in the water supply. The MATC is the highest level of Se that cannot be shown to be unsafe. For chinook salmon smolts, we defined it as the highest concentration of dietary or waterborne Se for which there is no detectable effect on either the normal development of the parr-to-smolt transformation during freshwater rearing, or their ability to adapt to sea water, or their ability to continue to grow and develop normally at salinities of 28-30 parts per thousand (ppt).

We assessed effects on juvenile chinook salmon by exposing them to Se in the diet or in the water supply and then monitoring their survival and growth in freshwater and in seawater, by performing gross anatomical investigations and histological examinations of selected tissues, by performing seawater challenge/plasma sodium tests and evaluating gill sodium/potassium adenosine triphosphatase (Na^+/K^+ ATPase) levels, by testing their ability to withstand stress-challenge, and by evaluating their performance in volitional out-migration tests.

Biological samples collected at Kesterson Reservoir were found to contain high concentrations of Se, some of which is probably present as organic selenium (Saiki, 1986). However, the full range of different Se-containing compounds, and the concentrations present in biological samples to which migrating salmon might have dietary exposure, have not been determined. In order to test the toxicity of organoselenium in the diet in a form and abundance that migrating salmon might encounter, we performed two dietary experiments: in one, we incorporated mosquitofish (*Gambusia affinis*) collected from the San Luis Drain at Kesterson NWR into a diet which we fed to the smolting chinook salmon, and in the other, we included pure selenomethionine in their diets.

Water samples collected from the San Luis Drain at Kesterson NWR had high concentrations of Se, molybdenum (Mo), and boron (B) (Saiki, 1986). We investigated the toxicity of Se in solution both with and without added Mo and B.

Methods

We obtained fall chinook salmon from the Washington State Department of Fisheries hatcheries at Soleduc (1985) and Minter Creek (1986-1989). After transporting the fish to the Marrowstone Field Station (MFS), Nordland (Washington) of the National Fisheries Research Center-Seattle, we treated them with formalin and TM-50 to control subclinical parasites and bacterial infections, and placed groups of fish in fresh water in experimental tanks. We fed them Oregon Moist Pellet (OMP) at standard hatchery feeding rates throughout the freshwater and seawater portions of the experiments. Se exposures were conducted in fresh water at less than 4 pounds fish per gallon per minute loading capacity and with water quality characteristics of hardness 74 ppm, alkalinity 74 ppm, pH 8.1, and at 10-13 degrees C, with dissolved oxygen near saturation.

I. Dietary exposures:

A. Using mosquitofish from the San Luis Drain.

For 7 weeks in 1985 we fed five groups of chinook salmon parr different amounts of an OMP diet containing organoselenium. We prepared the diet by incorporating Se-contaminated mosquitofish collected from the San Luis Drain at Kesterson NWR into an OMP formulation; mosquitofish composed 16% of the diet. Three test groups of fish received one, two, or four feedings per day of the Se-containing diet. These regimens resulted in test groups of fish receiving diets containing 6.5, 13, and 26 ppm Se (μg/g diet, wet weight). Due to limited supplies of uncontaminated mosquitofish, the remainder of the daily ration fed to the test groups consisted of a standard OMP diet containing no added mosquitofish. Two controls were incorporated in the experimental design -- a positive control (0.7 ppm Se) consisting of an OMP diet incorporating uncontaminated mosquitofish purchased from a commercial fish farm, and a negative control (0.5 ppm Se) consisting of the standard OMP diet with no added mosquitofish. All five groups were fed at the rate of 3% of body weight per day. Thus they were offered the same total amount of feed.

In 1986, we made an experimental diet by including 20% mosquitofish from the San Luis Drain in the OMP formulation. We made a control diet by adding 20% uncontaminated mosquitofish to the OMP recipe. We blended the two to give the desired final concentrations of 0, 3.3, 6.5, 13, and 26 ppm Se (μg/g diet, wet weight), and fed them to duplicate groups of fish at 3% per day for 7 weeks.

B. Using pure selenomethionine.

In 1986, we made test diets by adding seleno-D,L-methionine (Sigma Chemical Co.) to the OMP - uncontaminated mosquitofish control diet to obtain a final concentration of 26 ppm Se. We mixed control and experimental diets to make test diets and fed 0, 3.3, 6.5, 13, and 26 ppm (μg/g diet, wet weight), to duplicate groups of smolting chinook salmon.

In 1987 we again added selenomethionine to the control diet, obtaining a final concentration of 60 ppm Se in the experimental diet. Control and experimental diets were then mixed to make test diets with final levels of 0, 15, 30, and 60 ppm (μg Se/g diet, wet weight), which we fed to duplicate groups of fish.

II. Waterborne exposures:

In 1985 we exposed duplicate groups of chinook salmon to a chemical solution containing Se, Mo and B. The ratio of Se to Mo to B was 2:3:77, corresponding to an analysis of water from the San Luis Drain. The Se component contained +6 and +4 oxidation states in a 6:1 ratio. We added selenite as Na_2SeO_3, selenate as Na_2SeO_4, molybdate as Na_2MoO_4, and borate as H_3BO_3, and used an apparatus described by Mount and Brungs (1967) to deliver a 16-fold range of concentrations to test groups of fish for 32 d in fresh water. Then we acclimated the fish to sea water with no added Se and held them for an additional 30 d.

In 1986 we exposed duplicate groups of 200 juvenile chinook salmon to 0 (control), 9, 18, 35, 70, and 140 ppb (μg/L) Se, and in 1988 we exposed duplicate groups of 300 fish to 0 (control), 35, 70, 140, 280, and 560 ppb Se. As before, the test solutions contained +6 and +4 oxidation states of Se in a 6:1 ratio, derived from sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) respectively, but no Mo or B. We used a continuous-flow diluter (Garten, 1980) to deliver the appropriate concentrations of Se to the fish for 7 weeks (in 1986) or 30 d (in 1988) in fresh water. Then we acclimated the fish to sea water with no added Se and held them for an additional 3 months.

III. Evaluation of effects of dietary and waterborne Se:

A. Freshwater growth and mortality. In the 1985 dietary study, we individually weighed and measured 20 fish before and after Se exposure, and kept them separate from the rest of the population in a cage within the larger experimental tank. In all subsequent experiments we monitored freshwater growth by uniquely freeze-branding groups of 20 fish, which we then weighed and measured and released into each of the test populations before the Se exposure period. We recovered the 20 branded fish in each test and control group after the Se exposure for determination of growth. Daily mortality was recorded.

B. Histological examinations. We took tissue samples periodically during the Se exposures, and at intervals after conversion to sea water. We examined gill, pseudobranch, liver, kidney, pancreas, digestive tract, thymus, brain, olfactory tract and spinal cord tissues. We sectioned paraffin-embedded tissues and stained with hematoxylin and eosin and May-Grünwald Giemsa (Yasutake and Wales 1983). Selected tissues were stained with modified Gram stain (Brown and Hopps 1973) as necessary. We also stained selected tissues using the direct fluorescent antibody technique and/or Gram stain for the detection of bacterial kidney disease.

C. Osmoregulatory ability. The parr-smolt transformation is normally accompanied by a progressively increased ability by juvenile salmon to physiologically regulate levels of plasma sodium to less than 170 meq/L when challenged with 30 ppt sea water. Normally, pre-smolted salmon respond to a 24-h seawater challenge test with high plasma Na^+ concentration in survivors, and a high percentage of them die. As fish develop osmoregulatory competence, both mean plasma Na^+ concentration in survivors and percent mortality decline. We evaluated the effects of Se exposure on the development of normal osmoregulatory function by conducting periodic 24-h seawater challenges on

10-fish samples from each test and control group during the Se exposure. To conduct a test we transferred the fish from fresh water directly to full-strength sea water (28-30 ppt) and, after 24 h, counted the number of dead fish, took blood samples from survivors, and determined their plasma sodium concentrations by flame photometry (Clarke and Blackburn, 1977). The normal development of smoltification in salmon is also characterized by a progressive increase in the activity of gill Na^+/K^+ ATPase, an enzyme whose activity characteristically increases over the course of the parr-to-smolt transformation. We assessed the effects of Se exposure on the normal ATPase development pattern by taking gill tissues from 10-fish samples from each test and control group periodically both during the exposure, and afterwards, when the fish had been in sea water for 4 weeks. Gills were stored frozen and analyzed for Na^+/K^+ ATPase activity (Zaugg, 1982). Results are expressed as micrograms of inorganic phosphate released from adenosine triphosphate per milligram of protein in the gill tissue preparation per hour of incubation.

D. Stress challenge. To evaluate the effects of dietary organoselenium exposure on the ability of juvenile chinook salmon to tolerate stress, a necessary part of survival ability, we subjected test and control groups to a standardized crowding stress challenge test at a population density of 15 lbs/cubic foot (2 lbs/gal) for 5 h, followed by a 19-h recovery period. We took 10-fish blood samples at 0, 5, and 24 h and analyzed plasma for glucose and chloride concentration. For each test group we drew two physiological stress response curves describing the extent and duration of the resulting hyperglycemia and hypochloremia as a function of time after application of the stressor. We used the area under each curve as a semi-quantitative estimate of the severity of the stress response and the time needed for recovery; that is, as indicators of the ability of organoselenium exposed fish to withstand environmental stress.

E. Necropsy based fish health assessment. On selected occasions during the parr-to-smolt transformation we anaesthetized 10-fish samples from each replicate test and control group and measured individual weights and fork lengths. We took blood samples from the caudal artery into heparinized microhematocrit tubes, centrifuged them, determined the hematocrit, and analyzed the plasma with a refractometer to determine protein concentration. After gross examination of the eyes, gills, pseudobranchs and thymus, we cut away the body wall to expose the internal organs. Mesenteric fat, spleen, hind gut, kidney, liver, and mesentery were inspected and qualitatively evaluated. We tallied the results and compared groups (Goede, 1988).

F. Migratory tendency. Smoltification culminates in downstream migration and entry into the sea, where the young fish must survive, grow, and develop in order to contribute to the fishery. We measured the effects of freshwater Se exposure on migratory behavior by freeze-branding groups of 50-200 fish from each test and control population and transporting them to the U.S. Fish and Wildlife Service's Quilcene National Fish Hatchery for a volitional out-migration test. We placed three notched dividers in a standard 8-ft by 80-ft hatchery raceway at 20-ft intervals to form 4 pools, each 8 inches shallower than the preceding one. The water level in the deepest pool was 32 inches, and water flow ranged from 200-300 gpm. The branded fish were placed and confined with screens in the upstream end of the modified raceway. After 3-4 d acclimation period, we removed the restraining screen, allowing the branded fish freedom to migrate. Each day, fish were netted from the last downstream pool, identified by brand, and scored as migrants (Lam, 1985).

G. Seawater survival and growth. We evaluated the effects of exposing chinook salmon to Se in fresh water on their subsequent growth and survival in sea water over a 1- or 3-

month period. Fish were converted to full-strength sea water gradually, with 48-h intervals at 10 and 20 ppt salinity, followed by a holding period of 1-3 months in full-strength sea water without added Se for the duration of the experiment, to simulate the experience of fish leaving the Se-contaminated fresh water environment and entering the marine environment. A sample of 60-100 fish from each test and control group were anaesthetized and individually weighed at the start and end of the seawater holding period to evaluate growth. Daily mortality was recorded.

H. Tissue residues. Whole-body and liver concentrations of Se were determined in 10-fish samples from selected groups after exposure to Se in the diet or the water supply. Mo and B were also measured in 1985. Elements were quantified by atomic absorption spectrophotometry.

Principal Findings

I. Effects of dietary selenium Using Se-contaminated mosquitofish from the San Luis Drain:

Freshwater survival and growth. Survival was essentially 100% in groups of chinook salmon at the highest Se concentration tested, 26 ppm, in each of two experiments. We saw reduced growth in fresh water in fish fed test diets containing 26 and 13 ppm Se in each of the two experiments (Tables 1a, 1b). Fish receiving 6.5 ppm Se or less grew as well as the controls.

Histopathology. We documented several histopathologies whose frequency varied directly with the dosage of Se fed (Table 2). For instance, in the kidney, degenerative changes occurred in the endothelial cells of the glomerular capillary loop. This pathological change was most common in fish fed the test diet containing 26 ppm Se and was progressively less common at the lower dosages. Also, a subtle form of possible megalocytosis, usually characterized by an increase in the diameter of the hepatocyte and its nucleus (McCain et al, 1982), was found in the livers of 2 of 5 fish examined that received the same test diet and in 1 of 5 fish fed 13 ppm Se. None of the control fish examined had this condition.

Seawater challenge. In one experiment, mortality after 24-h seawater challenge was greatest in weeks 3, 4, and 5 (Table 3a) in chinook salmon fed 26 ppm Se, but there were no mortalities in week 6 at any level of dietary Se. In a second experiment, fish fed 26 ppm Se had higher percent mortalities than the controls during the seawater challenge in weeks 2 through 7 (Table 3b). These data suggest an inability to osmoregulate in fish receiving the diets containing 26 ppm Se.

Plasma sodium concentrations in survivors of the seawater challenge tended to be highest in fish fed diets containing 26 ppm Se at most of the time points in both experiments (Tables 3a, 3b), again indicating osmoregulatory dysfunction at the highest Se level tested. Plasma sodium levels in fish fed 13 ppm Se or less were not consistently higher than controls.

Gill Na^+/K^+ ATPase activity. Fish fed diets containing 26 ppm Se had the lowest mean gill Na^+/K^+ ATPase activities on all but one of six freshwater sampling dates in one experiment (Table 4a) and on every sampling date in fresh water in another experiment (Table 4b). After 4 weeks in sea water, no difference was detected between controls and fish fed 26 ppm Se, although fish fed a diet containing 13 ppm Se had markedly lower ATPase levels at that time. No reductions in gill ATPase activities were seen at other dietary levels.

Necropsy-based fish health assessment. The health assessment revealed that fish fed a diet containing 26 ppm Se were not growing as well as the fish receiving control diet (or any other test diet) as early as week 3. Fish fed 13 ppm Se were somewhat smaller after 3 weeks, when we first assessed health, and their reduced size persisted through week 7, when we ended the Se exposure (Table 5). Fish fed 26 ppm Se had a slightly elevated hematocrit after 3 weeks, a condition which worsened after 7 weeks on the test diet. The cause of the high hematocrit is unknown, but it may be related to dehydration due to impaired osmoregulatory capability. Other parameters measured in assessing health revealed no consistent trends at any dietary Se concentration.

Migratory behavior. There was an inverse relationship between dietary Se concentration and migratory tendency in one experiment, when we tested single populations at each dietary level (Figure 1). We saw no such relationship in a second experiment, when none of the groups migrated very readily, possibly due to the low water flows available that year.

Seawater survival and growth. All test and control groups survived at similar high levels (88-97%) and grew at comparable rates in one experiment. In the second experiment, survival in sea water was lower, due to an idiopathic mortality, and was not related to dietary Se concentration. All test groups grew at comparable rates in this experiment, although those fed 26 ppm Se while in fresh water were much smaller after 3 months in sea water than the controls (31.4 g *vs* 41.8 g). This reflects the reduced size of the fish at the end of the dietary exposure, a size difference which could not be overcome after 3 months in sea water.

Bioaccumulation. We saw a linear relationship between dietary Se level, and whole body concentration of Se (Table 6). On the other hand, the concentrations of Mo and B were not related to test group. Analysis revealed that the diets did not contain high concentrations of Mo and B.

II. Dietary effects of selenium using pure selenomethionine:

Freshwater survival and growth. There was no mortality attributable to selenomethionine level during the time test diets were fed to chinook salmon smolts in either of two experiments. Survival was close to 100% in groups of fish fed diets containing the highest Se concentrations, as high as 26 ppm for 42 d in one experiment, and 60 ppm for 30 d in the other.

Although the growth rate of chinook salmon fed a diet containing 26 ppm Se was only slightly reduced compared to the controls, fish were smaller in this group than in the controls after 42 d (Table 7a). In part, this reflects the slightly smaller size of the fish at the beginning of the growth experiment, due to the random assignment of fish to test groups. Usually, smaller fish tend to grow at a faster rate when other factors are equal, but this group failed to do so, presumably due to the effect of 26 ppm Se. There was no reduction in growth or size in any of the other test groups. Growth after 30 d was clearly reduced in fish fed diets containing selenomethionine at 60 ppm Se (Table 7b), and somewhat reduced at 30 ppm Se. At 15 ppm Se there was no clear reduction in growth.

Histopathology. The most consistent histopathological changes occurred in the pseudobranchs of the 60 ppm test group after 28 days. In the control fish, the epithelial and supporting pilaster cells were found in neatly arranged rolls, as is usual in normal tissue. In the pseudobranchs of fish receiving 60 ppm Se, most of the epithelial cells were hypertrophic and pleomorphic, giving the tissue a very irregular appearance. There were also many small foci of lymphoid cells with no apparent presence of bacteria or other pathogens. At the end of the dietary exposure, gill tissues in fish from the 60 ppm Se exposure group exhibited atypically large numbers of chloride cells. But after 28 d in sea water, chloride cell regression had occurred and gills from test and control fish appeared similar.

No significant histopathological changes were detected in the thymus, liver, kidney, or gastrointestinal tract.

Seawater challenge. In the first experiment, in which we tested diets containing 3.3-26 ppm Se, all groups suffered higher mortality than controls during 24-h seawater challenge as early as week 2 (Table 8a), but even fish fed 26 ppm Se were equivalent to the controls by week 4. It should be noted that this experiment was interrupted in week 5 by an epizootic, which may have contributed to elevated mortality in the control groups in weeks 4 and 5, thus complicating interpretation of results. In another experiment, after two weeks fish fed 15 ppm Se or more suffered higher mortality during seawater challenge than the controls (Table 8b). At 30 or 60 ppm, elevated mortality following challenge persisted through week 4, when we ended the exposures to the test diets. So inability to osmoregulate in chinook salmon was clearly indicated at dietary levels of selenomethionine of 30 ppm Se or more.

Plasma Na^+ concentrations in surviving fish suggested a similar interpretation. Seawater challenge survivors from the 26 ppm Se test group had higher plasma Na^+ concentrations than the controls from weeks 3-7 in the first experiment (Table 8a). Fish fed 13 ppm Se or less were about the same as the controls. Throughout the second experiment, fish fed 30 or 60 ppm Se consistently had higher plasma Na^+ levels after seawater challenge than did the controls (Table 8b), mirroring the mortality data. Fish fed 15 ppm Se could not be distinguished from the controls.

Gill $Na+/K^+$ ATPase activity. Gill ATPase activity generally reflected the osmoregulatory picture established by the seawater challenge test results. In the first experiment, fish fed 26 ppm Se had lower ATPase levels than the controls through most of the experiment, although they were even higher than the controls after the fish were off the Se-containing diet and in seawater for a month (Table 9a). At 13 ppm Se or lower, ATPase levels were about the same as the controls. In the second experiment, none of the fish developed a high level of ATPase activity, and only those fish fed 60 ppm Se had clearly and consistently lower ATPase levels than the controls (Table 9b).

Necropsy-based fish health assessment. The health assessment was only performed in 1986, when the highest Se level fed was 26 ppm. Mean fish weight was somewhat lower than controls in all test groups after 7 weeks (Table 10), although growth in general may have been distorted in this experiment, due to the epizootic in week 5 and the accompanying mortality, which may have masked the true effects. After 3 weeks (before the epizootic), the fish sampled from the 26 ppm Se test group were 13% smaller than the controls, supporting the suggestion of reduced size seen in the fresh water growth experiment. After 7 weeks, fish sampled from the 13 ppm Se test group were 16% smaller than controls, and those from the 6.5 ppm Se test group were 13% smaller. Other parameters in the assessment did not differ appreciably from the controls.

Migratory behavior. There were no differences in migratory behavior between the controls and any of the test groups in two experiments.

Stress challenge. Fish exposed to as little as 15 ppm Se in the diet appeared to be more susceptible to stress, as judged by the life-threatening hypochloremia that developed following challenge (Table 11). However, a strong hyperglycemic response did not occur and the effect of organoselenium exposure seems to be targeted on ionoregulation.

Seawater survival and growth. In the first experiment, survival in sea water was high in all test groups fed up to 26 ppm Se in the diet (Table 12a). In this experiment, control fish suffered the highest mortality in sea water, which was probably related to the epizootic in fresh water in week 5 of that experiment. In a second experiment, only 16% of the fish fed 60 ppm Se survived the first month in sea water (Table 12b). The other test groups also had somewhat lower survival than the controls in sea water.

All the fish that survived the first month in sea water grew at least as rapidly as the controls (Tables 12a, 12b). But after 3 months in sea water, survivors from the 60 ppm Se test group were still much smaller than controls.

Bioaccumulation. There was a direct relationship between levels of selenomethionine in the diet and both the whole body and liver concentrations of Se (Table 13). Liver concentrations were 3-4 times higher than whole body concentrations, suggesting that the Se-containing compounds were being deposited in the liver.

III. Effects of Se in the water supply:

Freshwater survival and growth. Survival in test groups of chinook salmon exposed to Se concentrations up to 560 ppb in the water supply for time periods from 34 d to 7 weeks ranged from 96 to 100%. In the presence of Mo and B at Se concentrations up to 20 ppb, survival was 100% after 34 d. Growth rates at these concentrations of Se for these time intervals were essentially equal for all groups. Thus, we saw no trend toward reduced survival or growth during exposure to Se in fresh water at concentrations up to 560 ppb.

Histopathology. We saw no histopathologies associated with levels of inorganic Se in the water supply up to 560 ppb in any of these experiments.

Seawater Challenge. There was no clear reduction in osmoregulatory capability, as measured by percent mortality after seawater challenge, nor by plasma Na^+ concentration in survivors of the seawater challenge at Se concentrations up to 20 ppb in the presence of Mo and B for up to 5 weeks.

Percent mortality after 24-h seawater challenge was weakly correlated with the Se concentration to which salmon were chronically exposed for 4 weeks and 7 weeks at concentrations up to 140 ppb (Table 14a). Fish exposed to 140 ppb Se in the water supply had the highest mortality on both occasions. But mean plasma Na^+ concentrations among survivors of the challenge was not similarly correlated. For no known reason, the plasma Na^+ concentrations were abnormally high for all groups.

When we repeated the experiment in 1988, testing concentrations from 35-560 ppb Se in the water supply, percent mortality after seawater challenge was lowest (0%) in stock fish sampled on May 11, one week before we started the Se exposures, and it increased thereafter (Table 14b). But plasma Na^+ levels were high on that date (192 meq/L), and at no time during the subsequent 8 weeks did they decline to the concentrations expected for fully functional salmon smolts (Table 14b). The percent mortality of all groups combined after 24-h seawater challenge was less than 10% in week 2. It rose to greater than 70% in week 5, and remained high thereafter, suggesting that parr-reversion had taken place.

The failure of the fish to demonstrate a normal pattern of development of osmoregulatory capability in both experiments in which the higher concentrations of Se in the water supply were investigated made it difficult to interpret the results of the seawater challenge experiments. In general, control fish tended to have the lowest percent mortality during the challenge. Some effect of inorganic Se in the water supply on the normal development of smoltification cannot be ruled out, although it was not convincingly demonstrated.

Gill $Na+/K^+$ ATPase Activity. In the presence of Mo and B, none of the Se exposure groups differed from controls, and all groups exhibited high gill Na^+/K^+ ATPase activities, as expected, from the earliest sampling date. We saw no evidence suggesting that exposure to the chemical solution for 32 d at Se concentrations up to 20 ppb had any effect on these ATPase activities.

In the experiment in which test concentrations ranged up to 560 ppb Se, with no added Mo or B, all groups exhibited the highest gill Na^+/K^+ ATPase activities on the first day of sampling, after 3 weeks exposure to Se, and they declined thereafter until the end of the Se exposure period (Table 15). Like the results of the seawater challenge experiments, these results suggested a loss in osmoregulatory ability in all groups including controls. Despite the apparent parr-reversion, all groups were physiologically able to adapt to sea water after gradual conversion. Thirty-five days after seawater entry, there was no indication in any test group of reduced ATPase activities as a result of Se exposure.

Migratory behavior. We saw no evidence of reduced migratory tendencies in any of the groups exposed to Se in the water supply, either in the presence of Mo and B at Se levels up to 20 ppb, or in their absence at Se levels up to 560 ppb.

Seawater survival and growth. When chinook salmon were exposed to Se in the water supply, survival of all test groups for 1-3 months in seawater was equivalent to that of controls in each of the three experiments. There was no evidence to suggest any effect of any level of Se tested on subsequent survival or growth in sea water.

Bioaccumulation. When we exposed salmon to a combination of Se, Mo, and B in a ratio of 2:3:77, at Se concentrations up to 20 ppb, we saw no tendency of the fish to accumulate any of the elements in their whole body tissues. Livers were not analyzed.

We saw a very slight tendency of juvenile chinook salmon exposed to Se in the water supply to accumulate Se in liver tissue, and none in whole body tissue, at any Se concentration up to 560 ppb (Table 16).

Summary

We studied the effects of adding Se-contaminated mosquitofish from the San Luis Drain to the diet of smolting chinook salmon. In one experiment, three test diets contained Se concentrations of 6.5, 13, and 26 ppm. In the other, four test diets contained Se concentrations from 3.3-26 ppm. Percent survival in fresh water in all test groups was equivalent to controls, so all effects were sublethal.

At 26 ppm Se, we saw clearly reduced growth in fresh water. During 24-h seawater challenge, at most time points in the two experiments we saw elevated percents of mortality, and survivors had elevated plasma Na^+ levels. Gill Na^+/K^+ ATPase activities lagged below the control levels at every time point in both experiments. The necropsy-based fish health assessment also reflected the reduced growth after 3 and 7 weeks. A single population of fish fed 26 ppm Se migrated less readily than the others in one of two experiments. Once they were off the Se-contaminated diets, and had successfully adapted to the marine environment, this test group grew at a faster rate than the controls, probably because they were smaller when they entered sea water. In spite of their faster growth rate, they were unable to attain the same size as the control fish after 3 months in sea water. Histopathologies in the kidney and liver were present in direct proportion to the Se dosage fed.

At 13 ppm, fish grew at a somewhat reduced rate in both experiments while they were in fresh water and receiving the test diets. The necropsy-based fish health assessment also reflected the reduced growth after 3 and 7 weeks at 13 ppm Se. A single population fish from this test group migrated less readily than the controls in one experiment. Gill Na^+/K^+ ATPase activities were comparable to controls in fresh water, but a month after the salmon entered sea water, we measured low gill ATPase activity in a single 10-fish sample from this test group. This result may be spurious since fish fed a higher dosage had ATPase activities equivalent to the controls at the same time. Histopathologies of the liver and kidney were directly related to Se dosage.

At 6.5 ppm Se we saw no effect on fresh-water growth or size while fish were receiving test diets. There was one instance of kidney histopathology in five fish examined. There was no compelling evidence for a negative effect of 6.5 ppm dietary Se on osmoregulatory capability, and no effects persisted into the early marine environment. Whole body Se concentration following dietary exposure was about 10 times higher than control fish. It is likely that the Se accumulated in the liver, as it did in our experiments with selenomethionine. There, the liver accumulated 3-4 times the Se concentration measured in the whole body. The consequences of a high concentration of Se in the liver on long-term growth and survival in sea water are unknown.

We concluded, based on the preponderance of our evidence, that at levels high enough to produce a final dietary concentration of 26 ppm Se, adding mosquitofish to the diet of smolting chinook salmon in fresh water clearly affected their growth and osmoregulatory ability, and possibly their migratory tendency. At 13 ppm Se, there appeared to be reduced growth and some osmoregulatory dysfunction. We saw evidence of reduced migratory tendency in fish fed 6.5-13 ppm Se. Based on these findings, we offer 3.3-6.5 ppm for 30 d as the highest dietary level of Se at which there are no detectable effects on smolting in chinook salmon.

It must be noted that we cannot absolutely attribute the observed effects to Se, since samples collected from the environment are complex, and are likely to contain a variety of substances which may be damaging. It is possible that some other component(s) carried by the mosquitofish may have caused the observed effects.

We studied the effects of adding selenomethionine to the diet of smolting chinook salmon at 4 concentrations (3.3-26 ppm Se) in one experiment and at 3 concentrations (15, 30, and 60 ppm Se) in another. There was less than 1% mortality in fresh water, while fish were receiving the test diets, so all effects were sublethal.

At 60 ppm Se in the diet we saw reduced growth in fresh water, impaired osmoregulatory ability (increased % mortality and elevated plasma Na^+ in survivors of the 24-h seawater challenge; reduced ATPase), histopathological changes, and hypochloremia after stress challenge. Liver Se level following the Se feeding period was highly elevated. Survival in sea water was much reduced during the first month, and although the surviving fish grew rapidly, they could not attain the same size as the control fish after 3 months in sea water.

At 30 ppm Se in the diet we saw reduced growth in fresh water, impaired osmoregulatory ability (increased % mortality and elevated plasma Na^+ in survivors of the 24-h seawater challenge), hypochloremia following stress challenge, reduced sea water survival, and elevated liver Se concentration.

Fish fed 26 ppm Se did not grow as large in fresh water. They suffered reduced and delayed development of osmoregulatory capability (increased % mortality and elevated plasma Na^+ in survivors of the 24-h seawater challenge; reduced gill ATPase activity). Ability to withstand stress challenge and liver Se accumulation were not measured.

At 15 ppm Se, fish suffered hypochloremia following stress challenge, reduced survival in sea water, and elevated liver Se concentration. Fish fed 15 ppm Se or less grew as fast as the control fish while they were being fed the diets, and appeared to suffer no residual effects when they were transferred to sea water.

At 13 ppm Se there was no difference in osmoregulatory capability detected by our tests, and no other differences were measured. Liver Se concentration, which was not measured, was probably somewhat elevated, as it was at 15 ppm.

Se clearly concentrated in the liver, so long-term liver damage is possible, but its consequences to survival in seawater are unknown. In general, we detected clear effects at 15-60 ppm Se. Based on these observations, we offer 6.5 ppm Se as the concentration of selenomethionine at which we detect no effect on smolting in juvenile chinook salmon.

We studied the effect of a solution containing Se, Mo, and B in a ratio of 2:3:77 at 5 Se concentrations up to 20 ppb on the smoltification and early marine survival of chinook salmon. We saw no effect at any concentration tested on any of the parameters investigated.

We assessed the effects on chinook salmon of dissolved Se concentrations from 9 to 140 ppb in one study, and from 35 to 560 ppb in a second study. Growth and survival in fresh water were comparable at all concentrations tested. Histological examination did not reveal pathological changes that were attributable to the treatments. We saw a weak correlation between Se concentration and mortality during seawater challenge on several occasions in both experiments, but plasma Na^+ concentration and gill Na^+/K^+ ATPase activities did not support these results. Interpretation of the results was complicated by the failure of the fish to demonstrate a normal pattern of development, so we were unable to fully assess the effect of waterborne Se on smoltification. We observed no decrease in migratory tendency, even after exposure to 560 ppb Se, and the survival and growth of all Se-exposed fish in seawater were comparable to controls. There was a small accumulation of Se in the liver of fish exposed to 560 ppb Se, the highest concentration tested.

Based on these experiments, we saw no clear evidence of a detectable effect of waterborne Se on the survival, growth, or development of juvenile chinook salmon, in fresh water or in sea water, either in the presence of Mo and B at Se levels up to 20 ppb, or in their absence at Se levels up to 560 ppb, the highest levels tested. We cannot rule out an effect on the development of osmoregulatory capability.

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Palmisano, A.P., and G.A. Wedemeyer. Tolerance of juvenile fall chinook salmon (Oncorhynchus tswatcha) to dietary intake of organoselenium during the parr-smolt transformation (In preparation -- intended outlet is Journal of Fish Biology).

Palmisano, A.P., and G.A. Wedemeyer. Tolerance of juvenile fall chinook salmon (Oncorhynchus tswatcha) to dissolved inorganic selenium exposure during the parr-smolt transformation (In preparation -- intended outlet is Journal of Fish Biology).

Table 1a. Fresh water growth of single populations of chinook salmon fed test diets containing selenium, added as selenium-contaminated mosquitofish from the San Luis Drain. The negative control fish received a diet containing no mosquitofish. The positive control fish received a diet containing uncontaminated mosquitofish. The test groups received a diet containing no mosquitofish when they were not fed the test diets.

Test group (ppm Se)	Mean Weight (g)		Increase (%)
	Day 0	Day 30	
0 (controls)			
Negative	4.4	6.9	57
Positive	4.0	5.7	42
6.5	4.2	6.1	45
13	4.2	5.6	33
26	4.3	5.5	28

Table 1b. Fresh water growth of chinook salmon fed test diets containing different concentrations of selenium-contaminated mosquitofish from the San Luis Drain for 48 d, beginning May 13, 1986. n is the number of replicate populations. Two replicate groups (3.3 and 13 ppm Se) were lost due to disease.

Test group (ppm Se)	n	Mean Weight (g)		Increase (%)
		Day 0	Day 30	
0 (controls)	2	3.5	11.4	232
3.3	1	3.2	10.5	229
6.5	2	3.4	11.0	231
13	1	3.5	10.7	203
26	2	3.5	8.3	133

Table 2. The number of salmon, in groups of 5 examined, suffering histopathological changes in the tissues indicated. Five fish were sampled from populations fed diets containing selenium, added as selenium-contaminated mosquitofish from San Luis Drain for 30 d in 1985.

Test group (ppm Se)	Kidney	Liver
Negative control	0	0
Positive control	0	0
6.5	1	0
13	3	1
26	5	2

Table 3a. Percent mortality (in parentheses, mean plasma Na+ concentration [meq/L] of survivors) of groups of 10 chinook salmon removed from test groups and subjected to 24-h seawater challenge. Fish were fed diets containing selenium-contaminated mosquitofish from the San Luis Drain beginning April 25, 1985, until tested.

	Exposure Period (weeks)				
Test group (ppm Se)	3 (May 15)	4 (May 22)	5 (May 28)	6 (June 4)	7 (June 11)
0 (controls)					
Negative	10 (193)	0 (180)	0 (174)	0 (175)	0 (162)
Positive	0 (205)	20 (183)	0 (181)	0 (180)	0 (167)
6.5	0 (204)	0 (181)	0 (164)	0 (185)	0 (159)
13	0 (201)	0 (181)	0 (177)	0 (189)	0 (170)
26	20 (208)	50 (184)	30 (200)	0 (186)	0 (172)

Table 3b. Percent mortality (in parenthesis, mean plasma Na+ concentration [meq/L] of survivors) of groups of 10 chinook salmon removed from each of tow replicate test groups and subjected to 24-h seawater challenge. Fish were fed test diets, starting May 13, 1986, containing selenium-contaminated mosquitofish from the San Luis Drain.

Test group (ppm Se)	Exposure Period (weeks)				
	3 (May 15)	4 (May 22)	5 (May 28)	6 (June 4)	7 (June 11)
0 (control)	20 (198)	15 (199)	20 (197)	5 (181)	0 (146)
3.3	10 (202)	15 (191)	5 (180)	0 (173)	0 (145)
6.5	5 (203)	0 (194)	5 (187)	0 (173)	0 (141)
13	5 (202)	5 (208)	5 (186)	10 (189)	0 (142)
26	25 (202)	20 (207)	20 (216)	20 (218)	5 (167)

Table 4a. Mean gill Na+/K+ ATPase activities (μmo Pi/mg prot/n) for chinook salmon fed diets containing selenium-contaminated mosquitofish from the San Luis Drain, beginning April 25, 1985. Ten fish samples were taken at weekly intervals from single populations on test diets.

Test group (ppm Se)	Exposure Period (weeks)					
	1	2	3	4	5	6
0 (controls)						
Negative	17.0	17.6	19.3	24.9	33.2	34.6
Positive	16.8	17.3	18.9	23.0	20.7	23.2
6.5	15.5	17.1	21.0	24.2	32.5	28.1
13	14.9	17.2	21.4	22.4	20.1	26.6
26	14.3	16.8	15.6	19.5	21.1	19.4

Table 4b. Mean gill Na+/K+ ATPase activities of smolting fall chinook salmon fed test diets beginning May 13, 1986 containing selenium-contaminated mosquitofish from the San Luis Drain. A sample of 10 fish was taken from each of 2 replicate test populations.

Test group (ppm Se)	Exposure Period (weeks)					
	2 (May 28)	3 (June 4)	4 (June 11)	5 (June 17)	7 (July 1)	7 (b) (July 31)
0 (control)	12.2	14.8	16.8	23.2	16.3	67.5
3.3	11.2(a)	15.4	23.1	20.9(a)	18.0	63.5(a)
6.5	10.8(a)	14.2	20.7	29.1	24.8	61.2
13	11.5(a)	14.6	17.2	20.9(a)	24.1(a)	50.1(a)
26	9.3(a)	11.2	13.5	12.8	12.1	65.1

(a) n = 1.

Table 5. Necropsy based fish health assessment. Fork length (F.L.), weight (wt.), and hematocrit (hct.) of 10-fish samples from duplicate groups of chinook salmon fed test diets containing selenium-contaminated mosquitofish from the San Luis Drain until sampled at 3 or 7 weeks in 1986. Coefficients of variation of the 20 fish combined are indicated in parentheses.

	Week 3			Week 7		
Test group (ppm Se)	F.L. (mm)	Wt. (g)	Hct. (%)	F.L. (mm)	Wt. (g)	Hct. (%)
0 (control)	88 (5.6)	8.1 (17)	47 (10)	97 (7.5)	12.1 (22)	48 (9.6)
3.3	87 (6.3)	7.8 (21)	47 (11)	100 (4.8)	11.9 (16)	51 (8.1)
6.5	86 (7.0)	7.6 (21)	43 (8.8)	99 (7.5)	12.1 (16)	49 (10)
13	83 (8.6)	7.3 (24)	45 (8.9)	97 (4.0)	11.1 (14)	45 (7.1)
26	79 (6.7)	5.5 (23)	50 (25)	86 (9.6)	8.0 (27)	59 (15)

Table 6. Bioaccumulation of selenium (Se), molybdenum (Mo), and Boron (B), expressed in micrograms per gram dry weight of whole body tissue in chinook salmon fed diets containing mosquitofish from the San Luis Drain for 33 days in 1985. Concentrations of Mo were below detectable limits, and of B were near detectable limits at all dietary levels.

Test group (ppm Se)	Whole Body Residues		
	Se (µg/g)	Mo (µg/g)	B (µg/g)
0 (controls)			
Negative	1.1	<0.9	8.2
Positive	1.0	<0.9	4.5
6.5	9.6	<0.9	<4.6
13	13	<0.9	9.3
26	23	<0.9	<4.6

Table 7a. Growth in fresh water of chinook salmon fed test diets containing selenium, added as selenomethionine, for 42 days beginning May 13, 1986. Twenty branded fish from each replicate test group were weighed at the beginning and end of the dietary exposures. Two groups (control and 13 ppm) were lost due to disease.

Test group (ppm Se)	n	Mean Weight (g)		Increase (%)
		Day 0	Day 42	
0 (controls)	1	3.6	10.3	186
3.3	2	3.4	10.2	200
6.5	2	3.6	10.3	186
13	1	3.4	10.2	200
26	2	3.2	9.0	181

Table 7b. Fresh water growth of fall chinook salmon fed test diets containing selenium, added as selenomethionine, for 30 days beginning May 5, 1987. n is the number of independent groups used in determining growth. Twenty branded fish from each replicate group were weighed before and after the dietary Se exposure.

Test group (ppm Se)	n	Mean Weight (g) Day 0	Mean Weight (g) Day 30	Increase (%)
0 (control)	4	4.1	10.8	164
15	2	4.0	10.4	159
30	2	4.0	9.4	139
60	2	3.9	7.5	91

Table 8a. Percent mortality (in parentheses, mean plasma Na+ concentration [meq/L] of survivors) of chinook salmon subjected to 24-h seawater challenge. Fish were fed diets containing selenomethionine, beginning May 13, 1986. Ten fish were sampled from each of two replicate test populations and challenged. Two groups (control and 13 ppm) were lost due to disease.

Test group (ppm Se)	Exposure Period (weeks) 2 (May 28)	3 (June 3)	4 (June 10)	5 (June 16)	7 (July 1)
0 (controls)					
Negative	0 (201)	5 (185)	10 (184)	20 (182)	0 (151)
Positive	10 (196)	0 (192)	0 (184)	0 (165)	0 (148)
6.5	20 (202)	0 (193)	0 (177)	0 (184)	0 (161)
13	20 (200)	10 (191)	0 (176)	10 (179)	0 (153)
26	35 (209)	20 (208)	30 (197)	10 (196)	0 (162)

Table 8b. Percent mortality (in parentheses, mean plasma Na+ concentration [meq/L] of survivors) of chinook salmon subjected to 24-h seawater challenge. Ten fish were sampled from each of 2 replicate test populations (there were four replicate control groups) and challenged. Fish were fed diets containing selenomethionine, beginning May 5, 1987.

Test group (ppm Se)	Exposure Period (weeks) 1 (May 13)	2 (May 19)	3 (May 26)	4 (June 2)
0 (control)	5 (184)	2 (168)	0 (163)	2 (158)
15	0 (182)	10 (164)	0 (163)	0 (160)
30	20 (187)	10 (200)	20 (185)	10 (173)
60	20 (218)	10 (212)	30 (216)	40 (210)

Table 9a. Mean gill Na+/K+ ATPase activities of chinook salmon fed diets containing selenomethionine beginning May 13, 1986. Ten fish were sampled from each of two replicate control populations at weekly intervals. Two groups (control and 13 ppm) were lost due to disease in week 5. Fish sampled on July 31 were fed test diets for 7 weeks, and then converted to seawater and fed OMP without added selenium for 4 weeks.

Test group (ppm Se)	Exposure Period (weeks) 2 (May 28)	3 (June 4)	4 (June 11)	5 (June 17)	7 (July 1)	7 (a) (July 31)
0 (control)	12.3	19.0	20.4	22.7	19.9	39.8
3.3	13.8	15.0	19.8	28.0	23.4	54.4
6.5	14.6	16.0	20.7	23.9	18.1	48.4
13	13.1	15.3	23.5	21.2	21.6	46.8
26	14.3	14.3	16.9	21.9	12.1	54.5

Table 9b. Mean gill Na+/K+ ATPase activities of 10-fish samples from replicate test groups of chinook salmon fed diets containing selenomethionine, beginning May 5, 1987.

	Exposure Period (weeks)				
Test group (ppm Se)	1 (May 12)	2 (May 19)	3 (May 26)	4 (June 1)	4 (a) (July 7)
0 (control)	8.6	9.0	9.5	12.3	18.9
15	7.5	7.1	10.5	11.4	19.8
30	7.8	7.9	10.3	10.7	21.5
60	7.8	4.2	8.0	6.0	(b)

(a) Fish sampled on July 7 were fed test diets for 4 weeks, and then converted to seawater and fed OMP without added selenium for 5 weeks.

(b) No live fish remaining.

Table 10. Fork length (F.L.), weight (wt.), and hematocrit (hct.) of 10-fish samples from duplicate groups of chinook salmon fed test diets containing selenomethionine until sampled after 3 or 7 weeks in 1986. Coefficients of variation of the 20 fish combined are indicated in parentheses.

Test group (ppm Se)	Week 3 F.L. (mm)	Week 3 Wt. (g)	Week 3 Hct. (%)	Week 7 F.L. (mm)	Week 7 Wt. (g)	Week 7 Hct. (%)
0 (controls)	86 (6.1)	7.6 (20)	50 (8.7)	99 (6.4)	11.6 (23)	46 (5.8)
3.3	85 (6.3)	7.4 (22)	49 (9.4)	97 (5.5)	10.8 (17)	50 (8.9)
6.5	86 (5.8)	7.2 (21)	48 (8.1)	97 (5.7)	10.1 (20)	48 (11)
13	86 (7.8)	7.6 (27)	47 (4.2)	94 (7.4)	9.7 (24)	50 (9.8)
26	82 (11)	6.6 (32)	47 (11)	96 (4.9)	10.3 (17)	50 (8.8)

Table 11. Effect of selenomethionine on physiological stress response of fall chinook salmon subjected to standardized crowding challenge at the indicated fish densities for a 5-hour period. Fish had been fed test diets for 4 weeks in 1987. A life threatening hypochloremia developed in the 15 ppm test groups.

Test group (ppm Se)	Mean loading density (0 to 5 hrs) (lbs/gallon)	Mean loading density (5 to 24 hrs) (lbs/gallon)	Percent increase relative to controls: Hyperglycemia	Percent increase relative to controls: Hypochloremia
0 (control)	1.9	0.09	100	100
15	1.9	0.10	103	138
30	1.8	0.09	102	121
60	1.5	0.07	100	116

Table 12a. Effect of dietary selenomethionine on subsequent survival and growth in seawater. We fed test diets to replicate groups of chinook salmon for 7 weeks in 1986. Then we weighed the fish individually, replaced the test diets with OMP without added selenium, and held the fish in seawater for 3 months. Almost all the mortality occurred in the first month.

Test group (ppm Se)	Survival (%)	Mean Weight (g)		Increase (%)
		Outset	After 3 months	
0 (controls)	51	10.1	32.6	223
3.3	97	10.1	40.0	297
6.5	99	10.1	39.8	295
13 (a)	98	10.6	39.6	275
26	98	9.5	40.0	317

(a) Only 1 replicate tank of fish survived the bacteria gill disease epizootic.

Table 12b. Effect of selenomethionine in the diet of chinook salmon on their subsequent survival and growth in sea water. We fed test diets to replicate groups for 30 days in 1987, then replaced the test diets with OMP without added Se. We weighed the fish individually before and after 3 months in sea water. All the mortality at 60 ppm Se, and almost all the mortality, occurred in the first month.

Test group (ppm Se)	Survival (%)	Mean Weight (g)		Increase (%)
		Outset	After 3 months	
0 (controls)	88	10.2	43.0	322
3.3	79	10.2	42.9	321
6.5	75	9.6	44.1	359
13	16	7.2	34.8	383
26	98	9.5	40.0	317

Table 13. Bioaccumulation of selenium in test groups of chinook salmon fed diets containing selenomethionine for 30 days in 1987. Results are expressed as micrograms of selenium per gram of dried tissue.

Test group (ppm Se)	Selenium Concentration ($\mu g/g$ dry wt)	
	Whole Body	Liver
0 (control)	0.95	2.7
15	12	36
30	24	76
60	34	140

Table 14a. Percent mortality (in parentheses, plasma Na+ concentration [meq/L] of survivors) of chinook salmon subjected to 24-h sea water challenge. Replicate groups exposed to inorganic Se in the fresh water supply beginning May 15, 1986. Ten fish were sampled from each of 2 replicate test groups and challenged.

Test group (ppb Se)	Exposure Period (weeks)	
	4 (June 13)	7 (July 3)
0 (control)	20 (206)	0 (185)
9	30 (212)	5 (193)
18	35 (217)	10 (202)
35	45 (224)	5 (188)
70	35 (216)	5 (200)
140	50 (209)	15 (190)

Table 14b. Percent mortality (in parentheses, mean plasma Na+ concentration [meq/L] of survivors) of chinook salmon subjected to 24-h seawater challenge. Replicate groups were exposed to inorganic Se in the fresh water supply beginning May 17, 1988. Ten fish were sampled from each of 2 replicate test groups and challenged.

Test group (ppb Se)	Exposure Period (weeks)					
	2 (May 30)	3 (June 5)	4 (June 13)	5 (June 20)	6 (June 26)	7 (July 4)
0 (control)	10 (187)	10 (204)	15 (206)	85 (221)	55 (213)	85 (212)
35	30 (197)	20 (200)	45 (200)	85 (225)	55 (217)	60 (211)
70	5 (196)	25 (201)	65 (207)	85 (220)	85 (199)	75 (214)
140	10 (194)	15 (194)	50 (210)	60 (257)	75 (210)	60 (212)
560	0 (202)	15 (199)	50 (209)	80 (230)	65 (205)	70 (214)

Table 15. Mean gill Na+/K+ ATPase activities at intervals of replicate groups of chinook salmon exposed to selenium in the fresh water supply. Data are means of 20-fish, 10 from each of 2 replicate test groups.

Test group (ppb Se)	Exposure Period (weeks)			
	1 (June 3)	2 (June 17)	3 (July 1)	4 (Aug 12)
0 (control)	13.5	9.5	6.5	30.3
35	15.3	9.2	7.3	36.1
70	13.7	9.7	6.8	30.2
140	13.3	8.8	7.5	34.7
280	16.0	8.2	7.1	31.3
560	16.7	9.6	7.4	29.5

(a) Fish sampled on August 12, 1988 were exposed to selenium in the fresh water supply for 7 weeks, then converted to and held in sea water without added selenium for 5 weeks.

Table 16. Bioaccumulation of Se by chinook salmon exposed to dissolved inorganic Se for 6 weeks beginning May 17, 1988.

Test group (ppb Se)	Selenium Concentration (μg/g dry wt)	
	Whole Body	Liver
0 (control)	2.1	4.5
35	1.6	---
70	1.6	5.2
140	1.4	---
280	2.5	5.7
560	2.2	8.7

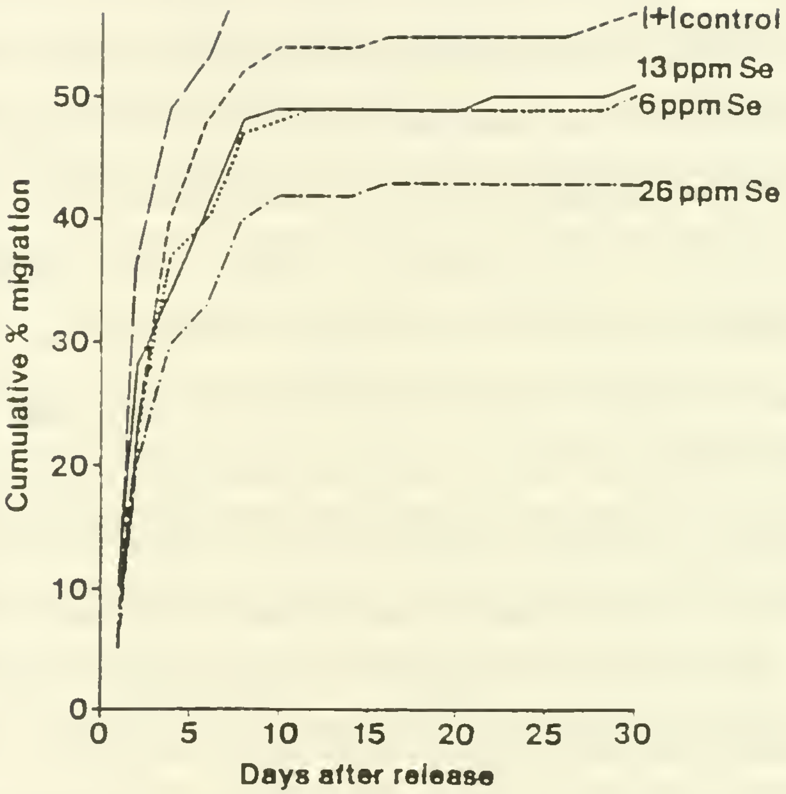

Figure 1. Cumulative percentage downstream migration, over 30 days, of fall chinook salmon fed different amounts of selenium–contaminated *Gambusia* in fresh-water for 34 days before they were released

ACUTE TOXICITY OF SELENIUM TO THE INVERTEBRATES _CHIRONOMUS RIPARIUS_ AND _DAPHNIA MAGNA_

Introduction

Recent surveys in and around Kesterson Reservoir (Kesterson) in the San Joaquin Valley of central California indicated that populations of fish, invertebrates, and wildlife species in this area are contaminated with selenium (Se) and several other elements (Saiki, 1986). Kesterson contained a series of 12 interconnected evaporation ponds that received flow from the San Luis Drain from 1978 to 1986. The San Luis Drain was constructed to convey subsurface drain water from irrigated fields in portions of the arid San Joaquin Valley and thus protect crops from salt damage and waterlogging of the soil. Intensive irrigation of these soils in the Valley has created a saline groundwater containing Se (average 300 μg/L; range 84 to 4,200 μg/L) and other potentially toxic metals that include arsenic, boron, chromium, copper, lead, mercury, molybdenum, vanadium, and zinc. In addition, the water in the Kesterson ponds contained from 60 to 390 μg Se/L. Contaminants (principally Se present in the drain water) have made Kesterson Reservoir a subject of concern because of the effects on fish and waterfowl. Plants and animals in these habitats have accumulated toxic concentrations of Se.

The discovery of Se toxicity in waterfowl at Kesterson, the proposal to extend the San Luis Drain to the Sacramento-San Joaquin Delta, and continued discharge of drainage water into the San Joaquin River and its tributaries resulted in efforts to determine the effects of Se from agricultural drainage to fish and aquatic invertebrates, and to develop alternative cleanup plans. Our objective in this study was to determine the acute toxicity of several forms of Se to the cladoceran _Daphnia magna_ and the midge _Chironomus_ riparius in a reconstituted fresh water representative of the San Joaquin Valley region of California.

Methods

Forty-eight hour acute toxicity tests were conducted with daphnids and midges with four Se preparations: (1) sodium selenate, (2) sodium selenite, (3) a 6:1 mixture of sodium selenate to sodium selenite (as Se), and (4) seleno-L-methionine (Sigma Chemical, St. Louis, MO). The 6:1 Se mixture represented environmental proportions of selenate to selenite found in the San Joaquin Valley region of California. Selenomethionine was chosen as a representative organic form of Se because its accumulation in animals is similar to that of naturally occurring organic forms of Se. Two dilution waters were used in the acute tests: (1) standard American Society for Testing and Materials (ASTM) soft water (hardness 40 to 48 mg/L as $CaCO_3$; alkalinity 30 to 35 mg/L as $CaCO_3$; sulfate 54 mg/L) and (2) a reconstituted fresh water representative of the San Joaquin River (SJR; hardness 134 mg/L as $CaCO_3$; alkalinity 60 to 65 mg/L as $CaCO_3$; sulfate 72 mg/L).

Seven test concentrations in a 60% dilution series were used in acute tests. Each test was started with 10 animals per test concentration. Midges were exposed individually in 25 ml of dilution water in 30-ml glass beakers. Daphnids were exposed together in 200 ml of dilution water in a 250-ml glass beaker. Mortality was recorded at 48 h. Death was defined as the lack of mobility in response to prodding with a blunt probe during a 5 s observation period. Daphnids were observed with the unaided eye and midges were observed at 250x magnification.

Principal Findings

Daphnids were more acutely sensitive than midges to the toxic effects of inorganic Se (Table 1). Selenate tended to be less toxic than either selenite or the 6:1 mixture of selenate to selenite. Seleno-L-methionine was extremely toxic to daphnids; mortality was 50 to 70% in 48 h at concentrations of 4 to 8 µg Se/L; however, no clear dose response was demonstrated (thus, no LC50 could be calculated). Seleno-L-methionine was acutely lethal to midges at concentrations about 1000 times greater than concentrations that were acutely toxic to daphnids. Daphnids tended to be more sensitive to Se in ASTM soft water than in harder SJR water, but no such trend was evident in the midge tests (Table 1).

Research Needs

Midges are generally perceived to be relatively insensitive organisms in toxicity testing. This conclusion is based on the practice of conducting short-term tests with 4th instar larvae, a procedure which may underestimate midge sensitivity to toxicants. In chronic toxicity tests started with first instar animals (Table 2), midges were sometimes as sensitive as daphnids to inorganic and organic compounds. In the present study, daphnids were more sensitive than midges to inorganic Se in acute (3- to 20-fold) and chronic (4-fold) testing. Although the sensitivity to inorganic Se forms was fairly similar between the two species, daphnids were about 1000 times more sensitive than midges to selenomethionine exposure. The reason for this dramatic difference in species sensitivity is unclear and requires further research.

In contrast to the extensive data base for inorganic Se, few data are available on the toxicity of organic Se compounds. We observed selenomethionine to be acutely lethal to daphnids at about 4 µg Se/L. If the ratio of acute to chronic toxicity of selenomethionine to D. magna is similar to that for inorganic Se (Table 2), the GM-MATC for chronic toxicity of selenomethionine to D. magna would be about 0.16 μg Se/L. Within the Kesterson Reservoir the concentration of organic Se forms reportedly represents up to a quarter of the total Se (Cooke, 1985). Although the USEPA Continuous Criterion Concentration of 5 μg Se/L for Se appears to protect invertebrates if only inorganic selenium exists, additional data are needed on the occurrence, chronic toxicity, and fate of organic Se compounds in fresh water before the hazard of Se in the aquatic environment can be adequately assessed.

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Ingersoll, C.G. 1989. Freshwater invertebrates accumulate levels of selenium that are potentially toxic to fish and waterfowl. U.S. Fish and Wildlife Service Research Information Bulletin No. 89-25.

Table 1. Selenium 48-h LC50 values (mg/L)[a] for *Daphnia magna* and *Chironomus riparius*.

Exposure medium	*D. magna*	*C. riparius*
San Joaquin River water		
Sodium selenate	4.07 (3.22-5.00)	16.2[b]
Sodium selenite	3.02 (2.48-3.76)	7.95 (5.21-14.5)
Inorganic selenium mixture[c]	2.62 (2.02-3.39)	9.34 (6.04-12.3)
Seleno-L-methionine	—[d]	5.78 (4.10-7.68)
ASTM soft water		
Sodium selenate	2.56 (2.02-3.22)	10.5 (7.91-13.4)
Sodium selenite	0.700[b]	14.6 (12.0-18.2)
Inorganic selenium mixture[c]	1.79 (1.39-2.33)	14.3 (12.3-17.0)
Seleno-L-methionine	—[d]	6.88 (5.30-8.87)

[a] 95% confidence intervals in parentheses.

[b] Confidence interval could not be calculated.

[c] Total selenium; a 6:1 selenate:selenite mixture.

[d] Dose response not determined.

Table 2. Chronic toxicity data for select compounds to midges and _Daphnia magna_.

Chemical	Type	Midge GM-MATC[a]	Daphnid GM-MATC[a]	Relative sensitivity[e]
Se, 6:1 mixture	Metaloid	504[b]	115	4.4
Toxaphene	Insecticide	2[c]	0.1	20.0
Kepone	Insecticide	12[c]	13	0.92
Fluorene	PAC[d]	417[b]	88	4.7
Fyrquel GT	Phosphate ester	48[c]	55	0.87
Kronitex 200	Phosphate ester	242[c]	13	19.0
Phosflex 31P	Phosphate ester	242[c]	40	6.1
Fluridone	Herbicide	848[c]	283	3.0

[a] Geometric Mean-Maximum Acceptable Toxicant Concentration (μg/L).

[b] _Chironomus riparius_.

[c] _Chironomus plumosus_.

[d] Polynuclear aromatic compound.

[e] MATC for _Daphnia_ divided by MATC for midge.

CHRONIC TOXICITY OF SELENIUM TO THE INVERTEBRATES DAPHNIA MAGNA AND CHIRONOMUS RIPARIUS

Introduction

Recent surveys at Kesterson Reservoir (Kesterson) in the San Joaquin Valley of central California indicated that populations of some fish and invertebrates in this area are contaminated with selenium (Se) and other trace elements (Saiki, 1986). Kesterson contained a series of 12 interconnected evaporation ponds that received flow from the San Luis Drain from 1978 to 1986. The San Luis Drain was constructed to convey subsurface drain water from irrigated fields in portions of the arid San Joaquin Valley and thus protect crops from salt damage and waterlogging of the soil. Intensive irrigation of these soils in the Valley has created a saline groundwater containing Se (average 300 μg/L; range 84 to 4,200 μg/L) and other potentially toxic metals that include arsenic, boron, chromium, copper, lead, mercury, molybdenum, vanadium, and zinc. In addition, previous studies at Kesterson NWR found the water in the ponds on the refuge to contain from 60 to 390 μg Se/L. Selenium concentrations in natural freshwater average about 1 μg/L. Contaminants (principally Se present in the drain water) have made Kesterson Reservoir a subject of concern because of the effects on fish and waterfowl. Plants and animals in these habitats have accumulated toxic concentrations of Se.

The discovery of Se toxicity in waterfowl at Kesterson resulted in efforts to determine the effects of Se from agricultural drainage to fish and aquatic invertebrates and to develop alternative cleanup plans. Our objectives in the present study were (1) to determine the chronic toxicity of a 6:1 mixture of sodium selenate to sodium selenite (as Se) to daphnids and midges in a reconstituted fresh water representative of the San Joaquin Valley region; and (2) to compare the relative sensitivity of several toxicity endpoints measured with these two species, including survival, growth, reproduction, or whole body ion concentrations.

Methods

Daphnids and midges were exposed in flow-through toxicity tests to the 6:1 selenate:selenite mixture (as Se) in 21- and 30-d life cycle tests, respectively. The dilution water was reconstituted San Joaquin River water. A modified Mount and Brungs proportional flow-through diluter and an automatic pipette (Micromedics^{R} Model 25000 PN) delivered five Se exposure concentrations and a control. Tests were started with either 10 daphnids or 50 midges introduced into each of four replicate 1-L glass beakers per exposure concentration. Flow splitting chambers (1-L Hefty^{R} freezer cartons) equipped with four 14-gauge aluminum hypodermic needles (Monoject^{R}) delivered 250 ml (±5%) of dilution water per cycle to each of the four replicate 1-L beakers per exposure concentration. Stainless steel screen (U.S. Standard sieve size #50, which retains first instar midges and daphnids) was used to cover a 4x13 cm overflow notch at the top of each beaker. Daily volume additions were 3.4 for daphnid and 6.0 for midge exposures. Nominal exposure concentrations (μg/L as total Se) were 1500, 750, 375, 188, 94, and 0 for the daphnid test and 6000, 3000, 1500, 750, and 0 for the midge test. Daphnid survival and reproduction (removal and counting of young) were monitored on Monday, Wednesday, and Friday each week. Wet and dry weights of daphnids surviving to the end of the exposure were recorded on day 21. Four approaches were used to estimate daphnid productivity: (1) day first gravid, (2) total number of young, (3) total young per available female reproductive days, and (4) the intrinsic rate of natural increase. Selenium Bioconcentration Factors (BCFs) for daphnids were calculated as the ratio of mean measured Se concentration in the daphnids to the mean measured Se concentration in the exposure water. Adult emergence was monitored daily in the midge exposures; cast pupal skins left by emerging adult midges were removed and counted daily. Day of first emergence, percent emergence, and mean emergence time of adult midges were measured for each beaker.

Principal Findings

Survival of daphnids was decreased only at the highest Se exposure concentration of 1410 μg/L during the 21-d exposure (Table 1). However, the day first gravid, total young, young per AFRD, and the intrinsic rate of natural increase (r) were all significantly reduced at Se concentrations ≥ 348 μg/L. The onset of reproduction (day first gravid) tended to be delayed at Se concentrations that reduced either reproduction or the intrinsic rate of natural increase. In addition, daphnid adult growth was significantly reduced at Se concentrations ≥ 156 μg/L. A stimulatory effect of Se on the size of offspring was observed at the two lowest exposure concentrations. Young produced by parents exposed to 85 or 156 μg Se/L were significantly larger than offspring from control parents. Whole body Mg, K, and Na concentrations in daphnids were not significantly affected by chronic Se exposure (Table 2). However, whole body Ca concentration significantly increased at intermediate Se exposure concentrations of 156 and 348 μg/L. In addition, whole body Cl concentration was significantly reduced at 711 μg Se/L. In daphnids exposed to increasing Se concentrations, Se accumulation increased and Se BCF decreased.

As in the acute tests, midges were less sensitive than daphnids to the toxic effects of Se in chronic exposures (Tables 1 and 3). Although percent adult emergence was reduced only at 6050 μg Se/L, the day of first emergence and emergence time were both significantly increased at Se exposure concentrations ≥ 837 μg/L (Table 3). The no observable effect concentrations (NOEC's) were 85 μg Se/L for daphnids and 303 μg Se/L for midges.

Research Needs

Waterborne inorganic Se is toxic to D. magna, C. riparius, and other invertebrates at levels greater than the recommended USEPA freshwater Criterion Continuous Concentration of 5 μg Se/L. Although dietary Se at or below expected environmental concentrations does not seem to be toxic to daphnids, inorganic waterborne Se can be biotransformed into organic Se compounds in the food chain. In the present study, daphnids accumulated 21 μg Se/g when they were exposed to a sublethal Se concentration of 85 μg/L for 21 d. Previous studies by our Center reported mean water concentrations of 122 μg Se/L in several Kesterson ponds and net plankton sampled from these ponds had accumulated 68 to 100 μg Se/g (dry weight, Saiki 1986). Our BCFs for daphnids were about one-tenth to one-third the values previously reported at similar water concentrations, possibly because additional dietary Se may be available to animals in the field. Selenium in the diet of fish and birds at about 5 to 10 μg/g may adversely affect growth, survival, development, or reproduction. In addition, Se in the diet of birds nesting at Kesterson NWR has been linked to adverse reproductive effects. Clearly, the bioaccumulation of Se by invertebrates either through the water or from the diet poses a toxic threat to fish and waterfowl through the food chain.

Ion regulation by fish is a sensitive indicator of sublethal exposure to many organic and inorganic contaminants. Contaminant stress may also impair ion regulation in invertebrates after short-term exposures. In the present study, changes in whole body ions were not sensitive indicators of Se toxicity to D. magna. Perhaps the animals acclimated to Se during the 21-d exposure, as has been shown in long-term exposures of fish and invertebrates. The only significant effect that we observed relative to the controls was an elevation in whole body Ca concentration at intermediate Se exposure concentrations (156 to 348 μg Se/L). Selenium reportedly interferes with Ca metabolism of both fish and invertebrates. As we observed in daphnids, Ca metabolism in amphipods is reportedly more sensitive than Na regulation as an indicator of contaminant stress. Sublethal metal exposure may cause little disruption of regulatory processes controlling monovalent ions in crustaceans; however, divalent ion regulation may be more severely affected by exposure to contaminants. Water quality characteristics and feeding may both influence daphnid whole body ion concentrations. Further research is required to better define the normal range of ion concentrations in D. magna and to determine the time course for changes in ion regulation by invertebrates resulting from exposure to contaminants.

If the ratio of acute to chronic toxicity of selenomethionine to D. magna is similar to that for inorganic Se (Work Unit #20027), then the predicted Maximum Acceptable Toxicant Concentration for seleno-L-methionine would be about 0.16 μg Se/L. Within the Kesterson Reservoir, waterborne organic Se reportedly represents up to a quarter of the total present (Cooke, 1985). Additional data are needed on the occurrence, chronic toxicity, and fate of naturally occurring organic Se compounds in fresh water before the hazard of Se in the aquatic environment can be adequately addressed.

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Table 1. Effects of chronic selenium exposure on mean survival, reproduction, and growth of Daphnia magna.[a]

Selenium[b] (μg/L)	n	Survival (%)	Day first gravid	Total young	Young/ AFRD[c]	r[d] (d^{-1})	Adult dry weight[e] (μg)	Body length of offspring (mm)
5(1.44)	4	92.5(4.15)A	8.0(0.35)A	1613(269)A	10.3(1.61)A	0.451(0.02)A	881(35)A	0.731(0.01)A
85(6.45)	3	93.3(5.44)A	9.0AC	1868(201)A	12.0(0.96)A	0.470(0.01)A	770(48)AB	0.764(0.01)B
156(10.0)	4	100.0A	8.5(0.43)A	1885(76)A	11.8(0.48)A	0.458(0.01)A	670(25)BC	0.772(0.01)B
348(21.3)	4	97.5(2.17)A	11.8(1.08)BC	858(126)B	5.4(0.79)B	0.402(0.01)B	567(36)CD	-
711(39.1)	3	93.3(2.72)A	11.0(1.63)AB	459(66)B	3.0(0.51)B	0.401(0.01)B	508(18)D	-
1410(85.1)	4	5.0(5.00)[f]	-	-	-	-	-	-

[a] Mean values (SEM in parentheses) sharing a common letter in each column are not significantly different ($p > 0.05$).

[b] Total selenium; a 6:1 selenate:selenite mixture.

[c] Total young per available female reproductive days (AFRD).

[d] Intrinsic rate of natural increase.

[e] Mean pooled individual dry weight per replicate.

[f] Not included in ANOVA.

Table 2. Effects of chronic selenium exposure to *Daphnia magna* on whole body ions and Se Bioconcentration Factors (BCF).[a]

Se[b]	Mg (μg/mg)	Ca (μg/mg)	K (μg/mg)	Na (μg/mg)	Cl (μg/mg)	Se (μg/g)	BCF
5	2.18(0.17)A	23.5(0.85)A	6.26(0.54)A	7.05(0.50)A	6.93(0.80)AB	1.3(0.1)A	322(24.8)A
85	1.84(0.15)A	22.2(1.19)A	7.81(0.74)A	7.05(0.61)A	6.90(0.64)AB	21.1(1.8)B	248(21.4)B
156	1.97(0.16)A	28.5(0.97)B	7.91(0.74)A	7.08(0.37)A	7.10(0.45)A	14.7(0.6)B	94(4.1)C
348	2.01(0.25)A	27.6(1.19)B	6.51(0.47)A	6.64(0.24)A	6.19(0.34)AB	31.7(3.0)C	91(8.7)C
711	1.58(0.04)A	23.3(1.49)A	7.31(0.49)A	6.09(0.44)A	4.83(0.28)B	51.1(3.0)D	71(4.2)C

[a] Mean values (SEM in parentheses) sharing a common letter in each column are not significantly different ($p > 0.05$). No samples were analyzed from animals exposed to 1410 μg Se/L.

[b] Total selenium; a 6:1 selenate:selenite mixture.

Table 3. Effects of chronic selenium exposure on *Chironomus riparius* emergence and emergence time.[a]

Selenium[b] (µg/L)	n	Emergence (%)	Emergence time (d)	Day first emergence
6(1.64)	4	88.5(6.02)A	16.3(0.17)A	14.0A
303(19.9)	4	90.0(4.06)A	16.6(0.30)A	14.3(0.22)AB
837(6.53)	4	86.5(2.77)A	17.6(0.06)B	15.0(0.35)BC
1384(31.3)	4	79.0(1.12)A	18.7(0.24)C	15.3(0.22)C
2953(53.6)	4	83.5(5.67)A	21.3(0.33)D	16.5(0.25)D
6050(91.9)	4	37.5(1.30)B	24.4(0.27)E	21.3(0.22)E

[a] Mean values (SEM in parentheses) sharing a common letter in each column are not significantly different ($p > 0.05$).

[b] Total selenium; a 6:1 selenate:selenite mixture.

ACUTE AND CHRONIC EFFECTS OF VARIOUS FORMS OF WATERBORNE SELENIUM ON JUVENILE STRIPED BASS

Introduction

Suisun Bay has been proposed as a possible receiving area for irrigation discharges from the Central Valley of California (Greenberg and Kopec, 1986). Because of the seleniferious characteristics of the soils in the Central Valley, the irrigation discharges are laden with potentially toxic concentrations of selenium (Se) and other contaminants (Saiki, 1986). Suisun Bay serves as habitat for important recreational and commercial species such as striped bass and chinook salmon (Greenberg and Kopec, 1986). However, the impact of contaminated irrigation discharges on the biota of receiving waters has not been adequately assessed. The National Fisheries Contaminant Research Center, through an intra-agency agreement with the Bureau of Reclamation, conducted studies to determine the toxicity and bioconcentration potential of Se in striped bass.

Methods

Acute toxicity studies: Acute toxicity studies were conducted in which striped bass were exposed for 96-hours to sodium selenate, sodium selenite, a 6:1 mixture of sodium selenate to sodium selenite, and seleno-L-methionine. Both a generic brackish water (described later) and standard ASTM soft water (hardness, 40-48 mg/L as CaCO3; alkalinity, 30-35 mg/L as CaCO3; pH, 7.2-7.6) was used to conduct the studies. The striped bass were exposed in 19.6 L glass jars that contained 15 L of the exposure waters. Treatments were spiked to obtain the desired exposure concentrations and there was a 60%

dilution factor between concentrations. Ten fish were placed into each jar and mortality was monitored every 24 hours.

Chronic toxicity studies: Sacramento River strain striped bass were obtained from the California Department of Fish and Game's Central Valley Fish Hatchery as 1-day old larvae. The fish were exposed in flow-through proportional diluters to a 6:1 mixture of sodium selenate to sodium selenite as Se for 90 days in two separate studies.

The experimental water was a generic brackish water representative of Suisun Bay and was prepared by blending appropriate amounts of Instant Ocean^{R} with reverse-osmosis (RO) water to yield a salinity of 1.2 g/L. Sodium bicarbonate was added to the water to increase its alkalinity to 70-75 mg/L as $CaCO_3$. The blended water was stored in 8,327 L polyolefin tanks for delivery by a pressurized system to the diluter. The studies were conducted at 18°C and the fish were fed a commercial diet three times daily.

Sodium selenate (98% pure, 41.8% Se) and sodium selenite (98% pure, 45.7% Se) stock solutions were prepared in RO water at concentrations corrected for percent purity and percent Se in the compounds. The stock solutions were prepared in brown glass containers and metered to the chemical mixing cell of the diluter separately with automatic pipettes. In the first study striped bass about 120-days old were exposed to concentrations of Se in the 6:1 mixture of 1.71, 0.86, o.41, 0.21, and 0.11 mg/L for selenate, and to 0.29, 0.14, 0.072, 0.036, and 0.018 mg/L for selenite, which constituted total Se concentrations of 2.0, 1.0, 0.5, 0.25, and 0.125 mg/L. In the second study fish about 80 days old were exposed to 2.57, 1.29, 0.64, 0.32, and 0.16 mg/L of selenate and 0.43, 0.21, 0.11, 0.05, and 0.03 mg/L of selenite, which constituted total Se exposure concentrations of 3.0, 1.5, 0.75, 0.375, and 0.188 mg/L. Fish were exposed in both studies to duplicate controls with no Se added.

In both studies, ten striped bass were placed into each small chamber of the exposure aquaria for growth determinations and 40 fish were placed in each large chamber for tissue residue analyses. Growth samples (total length and weight) were taken at 0, 30, 60, and 90 days of exposure and samples for Se tissue residue analysis were taken at 30, 60, and 90 days. Mortality in all treatments were recorded daily. The temperature, pH, hardness, conductivity, oxygen, chloride, and sulfate of the exposure water were determined weekly. The pH, alkalinity, chloride, and sulfates were measured on each blended tank of experimental water. One-hundred milliliters of exposure water were collected from mid-water column of each treatment and prepared for Se analysis at day 0 and every two weeks thereafter. Water samples for Se speciation were taken from the low, medium, and high concentrations at 0, 45, and 90 days. Refer to the section "Effects of Selenium on Bluegill Reproduction" for a description of the methods used to measure water quality and determine selenium in water and tissue samples.

Principal Findings

Acute toxicity studies: The acute toxicity of Se to striped bass in the ASTM soft water and in the 1.2 g/L saline water was similar (Table 1). In both waters selenate was less toxic than selenite. The toxicity of the 6:1 mixture of selenate to selenite was intermediate between that of selenate and selenite alone. The organoselenium compound seleno-L-methionine was more toxic to striped bass than the inorganic forms singly and in the 6:1 mixture. These studies show that the tolerance of striped bass to Se under acute exposure conditions is similar to that of bluegill.

Table 1. Ninety-six-hour LC 50's (mg/L) for striped bass exposed to various forms of Se in two different waters.

	Water Quality	
Selenium form	ASTM soft water	1.2 g/L saline water
Selenate	39.0	34.0
Selenite	1.0	6.0
6:1 Selenate:Selenite	15.0	18.0
Seleno-L-methionine	0.004	0.003

Chronic toxicity studies: In the chronic toxicity studies, survival and growth of striped bass were not affected by Se concentrations up to 3.0 mg/L. These results indicate that under chronic exposure conditions striped bass are more resistent to waterborne Se than bluegill which incurred significant mortality at concentrations of less than 1 mg/L Se . Thus tolerance of Se by fish may be species specific and/or influenced by water quality. The bioconcentration of Se by striped bass was directly related to exposure concentration, but Se was not highly accumulative in whole-body tissues of striped bass (Table 2). Bioconcentration factors ranged from 4 to 9.

Table 2. Selenium exposure concentrations, whole-body selenium concentrations, and bioconcentration factors for striped bass exposed for 90 days to a 6:1 mixture of sodium selenate to sodium selenite. Values are means and ± standard deviations.

Mean Se exposure concentration (μg/mL)	Mean whole-body Se (μg/g dry weight)	Bioconcentration factors
<0.006 (control)	0.811 ± 0.04	---
0.120 ± 0.015	1.12 ± 0.5	9
0.230 ± 0.006	1.57 ± 0.11	7
0.50 ± 0.06	2.31 ± 0.40	5
0.92 ± 0.03	4.70 ± 2.60	5
1.92 ± 0.068	8.26 ± 3.3	4

Research Needs

Determination of effects of water quality on Se toxicity: The studies conducted with striped bass showed that Se concentrations up to 3.0 mg/L were relatively non-toxic. whereas concentrations of less than 1 mg/L Se caused significant mortality and other adverse effects in bluegill. The difference in Se tolerance between striped bass and bluegill may be species specific or due to the effects of water quality on Se toxicity. Studies that determine the effects of water quality variables such as salinity on Se toxicity could be useful in predicting the impacts of Se discharges into saline receiving waters.

Determination of the effects of dietary Se on striped bass: Waterborne Se concentrations up to 3.0 mg/L were not toxic or highly accumulative in striped bass tissues: however, the effects of dietary and waterborne Se exposure together on striped bass need to be determined.

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ON-SITE TOXICITY OF SAN LUIS DRAIN WATER TO EARLY LIFE STAGES OF CHINOOK SALMON AND STRIPED BASS

Introduction

For more than 30 yr, agricultural subsurface (tile) drainage water from irrigated lands on the west side of the San Joaquin Valley, California, has flowed into the San Joaquin River (State Water Resources Control Board, 1987). Currently, over 30,800 ha of tile-drained lands contribute about 83,635,200 m^3 of drainage water to the river (State Water Resources Control Board, 1987). The tile drainage water contains dissolved (= non-filtrable) chromium, mercury, selenium, and other elements at concentrations approaching or exceeding the maximum limits recommended by the U.S. Environmental Protection Agency for protecting freshwater life (State Water Resources Control Board, 1987).

Recent contaminant surveys have shown that fish from several locations in the San Joaquin Valley contain elevated concentrations of arsenic, boron, cadmium, copper, mercury, lead, and selenium (Saiki and May, 1988). However, only selenium concentrations were seemingly influenced by inflows of tile drainage water into the San Joaquin River system, with highest concentrations of this element occurring in fish collected from reaches exposed to the drainage water. Whole mosquitofish from the San Luis Drain and Kesterson Reservoir (composed of a series of 12 interconnected evaporation ponds receiving inflows of tile drainage water from the Drain) have accumulated up to 370 $\mu g/g$ selenium on a dry weight basis, whereas whole green sunfish from Mud Slough--a west-side tributary of the San Joaquin River that flows adjacent to Kesterson Reservoir--have accumulated up to 23 $\mu g/g$. These concentrations greatly exceed 12 $\mu g/g$, the threshold limit for selenium toxicity that can elicit reproductive failure in sensitive species such as bluegills, largemouth bass, and other centrarchids.

Although reproductive studies with mosquitofish from the San Luis Drain documented low survival of fry when compared to mosquitofish from the Volta Wildlife Area (a nearby freshwater marsh that does not receive tile drainage, M.K. Saiki unpublished data), a cause-and-effect relation between high selenium concentrations and impaired reproduction in mosquitofish has not yet been verified. Moreover, although fish kills have been reported in the Drain and in tributaries of the San Joaquin River, the kills have generally been attributed to the misuse of agricultural pesticides or improper disposal of ammonia-based fertilizers rather than to toxic levels of elements in the tile drainage water (California Department of Fish and Game, unpublished data).

In the present study, we attempted to determine if prolonged (up to 28 days) exposure of juvenile chinook salmon and striped bass to actual tile drainwater resulted in measurable toxic effects. If the tile drainage water was toxic, we were also interested in determining if its toxicity was due to elevated salinity (which can approach 25-30 g/L), the unusual ratio of major ions composing the salinity (mostly Na_2SO_4 rather than NaCl as in ordinary seawater), or to "other" factors such as elevated levels of trace elements such as boron, molybdenum, and selenium.

Methods

Chinook salmon (40-50 mm total length) and striped bass (30-40 mm total length) fingerlings were obtained from the Merced River Fish Facility near Snelling, California, and the Central Valleys Fish Hatchery at Elk Grove, California, respectively. Both of these hatcheries are operated by the California Department of Fish and Game.

Experimental design: The chinook salmon and striped bass were exposed to serial dilutions (100%, 50%, 25%, 12.5%, and control) of tile drainage water (designated as

WWD), reconstituted drainwater (RWWD; contained approximately the same concentrations of major ions as WWD water but lacked elevated concentrations of elements), and reconstituted seawater (IO; 100% IO had approximately the same concentration of dissolved salts as did 100% WWD and 100% RWWD). All dilutions were made with water collected from either the San Joaquin River (SJR) at Crows Landing Road for the test with chinook salmon, or the Sacramento-San Joaquin Delta (DELTA) at the Tracy Fish Screen for the test with striped bass.

WWD water was collected from Sump #20 in the Westlands Water District. (Note: During the initial development of this study, we planned to test tile drainage water from the San Luis Drain. However, since July 1986, flows of tile drainage water into the San Luis Drain were prohibited. Thus, in order to conduct this study, we collected tile drainage water from an agricultural sump [Sump #20] that previously discharged into the San Luis Drain.) Based on ranked measurements of boron, molybdenum, selenium, and conductivity taken on eight occasions in 1986-1987, water from this sump was identified as the "worst" of 63 active sumps in the Westlands (Westlands Water District, unpublished data). Waters for RWWD and IO were formulated to meet the approximate concentrations of major ions (Ca^{+2}, K^{+}, Mg^{+2}, Na^{+}, Cl^{-}, HCO_3^{-}, and SO_4^{-2}) in WWD water and dilute seawater, respectively. Supplemental "controls" for the salmon tests included water collected from the Merced River Fish Facility (MR) and reconstituted river water (RSJR), whereas supplemental controls for the striped bass tests included water collected from the Central Valleys Fish Hatchery (EGH) and reconstituted delta water (RDELTA). Both RSJR and RDELTA waters were formulated so as to contain approximately the same concentrations of major ions as in SJR and DELTA waters, respectively.

Procedures: The tests were conducted as 28-day-long static tests, with daily renewal of the test solutions. New batches of water were either collected or reconstituted once every 4 days. At that time, water samples were collected for measurement of routine water quality parameters (i.e., bicarbonate, carbonate, and total alkalinity; calcium, magnesium, and total hardness; total dissolved solids; turbidity; ammonia; chloride; sulfate; and sodium). Additional samples were collected and preserved for analysis of trace elements. Temperature, dissolved oxygen, pH, and conductivity were measured daily in each experimental chamber. Water quality parameters are summarized in Tables 1-2.

(Note: In order to determine if the toxicity of tile drainage water varied among collection periods, supplemental 96-hr-long acute tests were conducted by exposing 1-day-old fathead minnow larvae to serial dilutions--100%, 50%, 25%, 12.5%, and control--of WWD, RWWD, and IO waters whenever new batches of water were collected or formulated. The dilutions were made with a standard EPA "very hard" reconstituted water. Preliminary results indicate that all fathead minnow larvae died in full-strength WWD water during each of the tests. Furthermore, survival of larvae was independent of exposure time at WWD concentrations ≥12.5%. The factors responsible for temporal variations in toxicity of WWD water are still being investigated.)

All fish were fed twice daily with BioDiet^{R}, once in the morning and again in the afternoon. Samples of BioDiet^{R} were saved for analysis of trace elements (boron, molybdenum, and selenium) by wrapping in polyethylene, then double-bagging and freezing at -10°C. Each tank was cleaned daily by siphoning off uneaten food and fecal material, then approximately 80% of the water was replaced.

The numbers of live and dead fish were counted daily, and dead fish were removed and saved for analysis of trace elements. On Day 0, all fish were measured for total length and weight. On Days 14 and 28, the surviving fish were remeasured in order to estimate growth rates. On Day 28, all remaining live and dead fish were saved for analysis of trace elements (boron, molybdenum, and selenium); these samples were prepared for storage by wrapping individual fish in polyethylene, then double-bagging and freezing them (-10°C).

Water temperature, dissolved oxygen, pH, and conductivity were measured daily in each experimental chamber. In general, the measured variables (Table 1) were within the acceptable limits for culturing juvenile chinook salmon and striped bass.

Water samples for measuring routine water quality parameters (i.e., bicarbonate, carbonate, and total alkalinity; calcium, magnesium, and total hardness; total dissolved solids; turbidity; ammonia; chloride; sulfate; and sodium) were obtained whenever new batches of each water type were either collected or reconstituted. These measurements, which were determined with standard methods (APHA et al. 1975), varied considerably according to water type (Table 2).

Additional water samples were collected for analysis of trace elements. These samples were filtered through 0.4 μm polycarbonated membranes, acidified to pH <2 with ultrapure nitric acid, then stored frozen (-10°C) in 500-mL polyethylene bottles until analyzed for boron, chromium, copper, molybdenum, and selenium.

Trace element analyses: All samples of water, fish food, and fish were analyzed by the National Fisheries Contaminant Research Center in Columbia, Missouri. Water samples were analyzed "as is", with no prior digestion. For determinations of chromium and copper, subsamples were drawn and diluted twofold with 20% nitric acid, then the analytes were measured by graphite furnace atomic absorption spectrophotometry. Zeeman background correction was needed in the analysis of chromium because of the high salt content of some samples; conventional deuterium arc background correction was used for the analysis of copper. Water samples were analyzed for boron and molybdenum by inductively coupled argon plasma spectroscopy, whereas selenium was analyzed by hydride generation atomic absorption spectrophotometry.

Fish food and fish samples were lyophilized to determine moisture content. Prior to analysis of boron, aliquots of the samples were subjected to a nitric acid-hydrogen peroxide microwave acid digestion. For analysis of molybdenum, the aliquots were digested with the nitric acid-hydrogen peroxide microwave acid procedure, but the resulting digestate was then preconcentrated by coprecipitation. Aliquots intended for selenium determination were subjected to a nitric acid-magnesium nitrate dry-ash procedure. Boron and magnesium were determined by inductively coupled argon plasma spectroscopy, whereas selenium was determined by hydride generation atomic absorption spectrophotometry.

Statistical analyses: Toxic effects on percent survival (arcsine-transformed values), growth, body condition, and whole-body concentrations of trace elements in fish (logarithmically transformed values) were evaluated by analysis of variance (ANOVA). Treatment means were compared against the control mean with Dunnett's one-tailed or two-tailed t tests. Pearson's product-moment correlation analyses were used to determine the relations between exposure concentrations, survival, growth, body condition, and whole-body concentrations of trace elements.

Principal Findings

Chinook salmon test: The survival of chinook salmon was affected by exposure to WWD water, but not to RWWD and IO waters (Figure 1). In addition, survival of salmon exposed to WWD water was affected only at the highest concentration of this water type (i.e., full-strength or 100% WWD). Mortalities first occurred in full-strength WWD water on Day 19 of the 28-day-long test, then progressed at a rate of about 1.6 fish/day through the end of the test.

The lengths and weights attained by chinook salmon were markedly affected by 28 days of exposure to both WWD and RWWD water, but not to IO water (Figures 2-3). In both WWD and RWWD waters, dose responses (i.e., significant correlations between lengths or weights attained by salmon and the concentrations of these two water types) were observed, with growth generally increasing as the concentrations of these waters were decreased by dilution with SJR water.

With one exception, chinook salmon held in the various treatments experienced appreciable growth during the test. Unlike fish exposed to other treatments, salmon held in full-strength WWD water exhibited negative growth (i.e., after 28 days, the survivors were slightly shorter and weighed less than fish on Day 0). In general, chinook salmon held in full-strength WWD water grew at a slightly slower rate than salmon held in full-strength RWWD water (Figures 2-3), suggesting that the WWD water was somewhat more toxic than the RWWD water.

The relative condition of chinook salmon exhibited a dose response in serial dilutions of WWD water, but not in serial dilutions of RWWD and IO waters (Figure 4). These data verify that salmon exposed to high concentrations of WWD water lost weight during the 28-day-long test. Our observations of the feeding activity of fish held in full-strength WWD water showed that they readily consumed food only during the first 8 days of the test; thereafter, these fish would "mouth" the food, but then spit the food out. During the final 7 days of the test, many fish held in full-strength WWD water were emaciated, and few showed any interest in feeding. In contrast, salmon held at lower concentrations of WWD water and at all concentrations of the other water types actively fed throughout the test.

Dissolved concentrations of five elements--boron, chromium, copper, molybdenum, and selenium--varied considerably according to water type (MR, IO, SJR, RSJR, WWD, and RWWD; see Table 3). Maximum concentrations (μg/L) of each element were as follows (water type given in parentheses): boron, 48,800 (WWD); chromium, 25.4 (WWD); copper, 13.2 (IO); molybdenum, 766 (WWD); and selenium, 218 (WWD). Only selenium concentrations exceeded the national criteria for protecting aquatic organisms and their uses (U.S. Environmental Protection Agency 1986, 1987). According the existing national criterion for selenium, the four-day average concentration in freshwater should not exceed 5.0 μg/L more than once every three years, whereas the four-day average concentration in saltwater should not exceed 71 μg/L (U.S. Environmental Protection Agency 1987).

The concentration (μg/g, dry weight) of elements accumulated by chinook salmon after exposure for up to 28 days to serial dilutions of WWD water generally increased as the concentration of WWD water increased (Table 4). In fish exposed to full-strength WWD water, boron averaged 228 μg/g, molybdenum averaged 0.404 μg/g, and selenium averaged 2.12 μg/g. In contrast, fish exposed to waters from "control" locations (e.g., MR; SJR) and from full-strength reconstituted sea water (IO) and full-strength reconstituted drainage water (RWWD) contained concentrations of boron $\leq$5.8 μg/g, molybdenum $\leq$0.33 μg/g, and selenium $\leq$1.7 μg/g. Approximate "background" concentrations of trace elements in whole freshwater fish are as follows: boron, <4 μg/g; molybdenum, <0.4 μg/g; and selenium, 1.8-2.3 μg/g (Saiki and May 1988). Except for the concentrations of boron in salmon exposed to full-strength WWD water, none of the elemental concentrations in fish were elevated above background concentrations. The toxicological significance of elevated boron concentrations in fish tissues is unknown.

Striped bass: The survival of striped bass was greatly affected by exposure to both WWD and RWWD waters, but not to IO water (Figure 5). In both WWD and RWWD waters, dose responses were evident; however, effects were more pronounced in WWD water than in RWWD water. In full-strength WWD water, all 20 fish were dead by Day 23 whereas, in full-strength RWWD water, 1 of 20 fish was still alive on Day 28.

Unlike the easily recognizable dose responses seen in survival data, the growth and relative condition of striped bass varied considerably (Figures 6-8), making detection of dose-response patterns difficult. Moreover, because all fish exposed to full-strength WWD water died by Day 23, no specimens from this treatment were measured on Day 28. Nevertheless, based on data from Day 14, striped bass held in WWD water seemingly exhibited a dose response for growth (weight only; Figure 7) and relative condition (Figure 8). Moreover, whereas fish held in other dilutions of WWD water and in all dilutions of other water types (RWWD and IO) exhibited measurable growth between Days 0 and 14, those held in full-strength WWD water failed to grow. We suspect that if any striped bass held in full-strength WWD water had survived the test, they would not have shown any appreciable growth. Striped bass held in RWWD water for 28 days also demonstrated dose responses for growth and relative condition, whereas those held in IO water showed no such responses (Figures 6-8).

Maximum concentrations (μg/L) of elements in full-strength WWD water were as follows: boron, 52,000; chromium, 34; copper, 6.5; molybdenum, 760; and selenium, 270 (Table 3). By comparison, maximum concentrations (μg/L) of elements in the other water types (EGH, RDELTA, DELTA, IO, and RWWD) were as follows: boron, 2,600; chromium, 32; copper, 12; molybdenum, 80; and selenium, 2.6. Only selenium concentrations exceeded the national criterion for protecting aquatic organisms and their uses (U.S. Environmental Protection Agency 1986, 1987).

Mean elemental concentrations (μg/g, dry weight) in whole striped bass held for up to 28 days in various dilutions of WWD water were as follows: boron, 2.25-136; molybdenum, 0.223-<0.309; and selenium, 1.26-1.47 (Table 4). Striped bass held in other water types for the same time period contained the following concentrations: boron, 2.15-<3.79; molybdenum, 0.180-<0.309; and selenium, 1.06-1.23. Except for boron concentrations in striped bass held in full-strength WWD water, none of the measured concentrations of elements exceeded typical background concentrations for whole freshwater fish (Saiki and May 1988). The toxicological significance of elevated boron concentrations in tissues of striped bass are unknown.

Relation of toxic endpoints to water quality: The toxic endpoints measured in fish during this study (i.e., survival, growth in total length and weight, and relative body condition) and physico-chemical water quality variables were assessed in order to discern any patterns. As judged by Pearson product-moment correlations, only a few associations were statistically significant ($P \leq 0.05$; Table 5). In general, toxic effects increased in severity (i.e., mortality increased; growth and body condition decreased) as dissolved salts and trace elements increased in concentration.

Conclusions and Research Needs

Tile drainage water was toxic to both chinook salmon and striped bass when fish were exposed for up to 28 days. The toxicity of tile drainage water was partly influenced by its concentrations of major ions because reconstituted drainage water--which contained a similar mixture of major ions but lacked elevated concentrations of trace elements--was also toxic to fish. However, the toxicity of tile drainage was somewhat greater than that of reconstituted drainage water, suggesting that other factors in tile drainage (e.g., elevated concentrations of trace elements; other unmeasured variables) also contributed to its toxicity. High salinity apparently did not contribute to the overall toxicity of tile drainage water because fish held in reconstituted seawater did not experience excessive mortality or exhibit reduced growth and body condition.

As judged by the concentrations of trace elements--boron, chromium, copper, molybdenum, and selenium--measured in tile drainage water, only selenium exceeded the national criteria for protecting aquatic life. However, only boron accumulated to excessive concentrations in fish held in full-strength tile drainage water.

To better understand the toxicity of tile drainage water, additional research is needed as follows:

1. The chronic toxicity of sodium sulfate (the predominant "salt" occurring in tile drainage water) should be characterized for chinook salmon and striped bass. Although the State of California has established water quality objectives for boron, molybdenum, selenium, and conductivity (a measure of the concentration of total dissolved solids) in the San Joaquin River and some tributaries (see State Water Resources Control Board 1987), there are no objectives for sodium sulfate. According to data compiled by McKee and Wolf (1963), some freshwater fishes cannot tolerate concentrations of sodium sulfate that exceed 100 mg/L.

2. The environmental factors influencing the accumulation of boron in fish must be further investigated. Both chinook salmon and striped bass held for up to 28 days in full-strength tile drainage water accumulated over 130 μg/g of boron, which is considerably higher than published values for whole freshwater fish. According to the U.S. Environmental Protection Agency (1986), there is no known nutritional requirement for boron in animals (including fish). Therefore, excessive accumulations of boron in fish tissues could represent the initial onset of a toxic reaction to this element.

3. The national criterion for selenium that is intended to protect freshwater aquatic life is 5 μg/L (U.S. Environmental Protection Agency 1987). During our study, dissolved concentrations of selenium in full-strength tile drainage water exceeded 100 μg/L. However, chinook salmon and striped bass exposed to these waterborne concentrations accumulated <2.2 μg/g of selenium, which is well within the background concentration for whole freshwater fish (Saiki and May 1988). Nevertheless, previous studies have demonstrated that the major route of uptake for selenium in fish is through the aquatic food chain (Lemly and Smith 1987). Thus, in order to better evaluate the potential threat from selenium toxicity, additional studies are needed where fish are fed forage organisms that have naturally accumulated selenium from the tile drainage water.

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Table 1. Summary of water temperature, dissolved oxygen concentration, pH, and conductivity in the experimental chambers. Values were geometric means (ranges in parentheses).[a]

Treatment[b]	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (µmhos/cm @ 25°C)
		Chinook salmon test		
WWD:				
100%	11.6 (11-14)	6.32 (5.3-8.1)	8.23 (8.1-8.6)	32,300 (30,000-37,000)
50%	11.5 (11-14)	5.36 (3.2-8.9)	8.17 (8.0-8.7)	19,700 (18,000-24,000)
25%	11.4 (11-14)	5.73 (3.7-9.5)	8.02 (7.8-8.6)	9,030 (7,900-16,000)
12.5%	11.4 (11-13)	5.53 (3.3-9.8)	7.92 (7.6-8.6)	5,910 (5,000-7,200)
RWWD:				
100%	11.4 (11-13)	4.87 (2.9-8.0)	7.53 (6.8-8.2)	26,100 (22,000-30,000)
50%	11.4 (11-14)	4.86 (2.7-8.9)	7.71 (7.4-8.4)	15,800 (9,900-19,000)
25%	11.5 (11-14)	5.57 (3.5-9.5)	7.81 (7.5-8.5)	7,490 (6,200-9,100)
12.5%	11.5 (11-14)	5.59 (3.8-9.7)	7.77 (7.2-8.5)	5,020 (4,000-6,000)
IO:				
100%	11.5 (11-14)	4.60 (2.6-8.1)	7.82 (7.5-8.1)	33,100 (28,000-38,000)
50%	11.5 (11-15)	5.26 (3.1-8.9)	7.79 (7.4-8.3)	19,600 (15,000-26,000)
25%	11.5 (11-15)	5.53 (3.5-9.6)	7.80 (7.5-8.3)	9,210 (7,400-16,000)
12.5%	11.5 (11-15)	5.54 (3.8-9.8)	7.81 (7.4-8.4)	5,780 (4,600-7,800)
Controls:				
SJR	11.4 (11-14)	5.91 (3.8-9.8)	7.83 (7.4-8.5)	2,230 (1,500-3,100)
RSJR	11.4 (11-14)	5.62 (3.9-8.8)	7.67 (7.5-8.3)	2,270 (1,900-2,600)
MR	11.4 (11-13)	6.13 (4.1-10)	6.53 (6.1-8.4)	63.1 (54-84)
		Striped bass test		
WWD:				
100%	18.4 (17-23)	5.17 (3.6-7.9)	8.19 (8.0-8.4)	27,100 (25,000-30,000)
50%	18.3 (17-22)	4.88 (2.6-7.1)	8.00 (7.0-8.2)	11,700 (9,100-18,000)
25%	18.3 (17-22)	4.83 (2.5-7.4)	7.82 (7.7-8.0)	6,220 (5,600-6,900)
12.5%	18.3 (17-22)	4.83 (3.0-7.3)	7.65 (7.4-8.6)	3,860 (3,400-4,600)

Table 1. (Continued)

Treatment[b]	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (µmhos/cm @ 25°C)
		Striped bass test (Cont'd)		
RWWD:				
100%	18.5 (17-23)	4.51 (2.7-7.9)	7.74 (7.4-8.3)	26,100 (23,000-28,000)
50%	18.4 (17-22)	4.84 (2.9-7.2)	7.57 (7.4-7.8)	10,300 (9,200-18,000)
25%	18.3 (17-22)	5.31 (3.6-7.5)	7.51 (7.4-7.8)	6,060 (5,800-6,400)
12.5%	18.3 (17-22)	4.65 (2.8-7.4)	7.46 (7.3-8.4)	3,750 (3,400-4,200)
IO:				
100%	18.3 (17-22)	4.27 (2.7-6.3)	8.13 (7.7-8.4)	27,700 (26,000-30,000)
50%	18.3 (17-22)	5.31 (3.6-7.2)	7.98 (7.2-8.3)	11,200 (9,400-18,000)
25%	18.3 (17-22)	5.15 (3.5-7.4)	7.81 (7.5-8.1)	6,150 (5,800-6,600)
12.5%	18.3 (17-22)	5.04 (3.5-7.6)	7.63 (7.4-7.9)	3,670 (3,400-4,000)
Controls:				
DELTA	18.3 (17-22)	5.30 (3.7-7.5)	7.32 (7.0-7.6)	659 (500-900)
RDELTA	18.3 (17-22)	4.91 (2.7-7.4)	7.28 (7.0-7.5)	580 (350-890)
EGH	18.4 (17-22)	5.08 (3.2-7.6)	7.67 (7.4-7.9)	380 (360-410)

[a] Sample size for the chinook salmon test, N = 29; for the striped bass test, N = 28.

[b] WWD, tile drainage water from Sump #20 in the Westlands Water District; RWWD, reconstituted drainage water; IO, reconstituted seawater; SJR, water from the San Joaquin River at Crows Landing Road; RSJR, reconstituted river water; MR, water from the Merced River Fish Facility; DELTA, water from the Sacramento-San Joaquin Delta at the Tracy Fish Screen; RDELTA, reconstituted delta water; and EGH, water from the Central Valley's Fish Hatchery.

Table 2. Concentrations of selected major ions and related physico-chemical variables in various water types used during the chinook salmon and striped bass tests. $\bar{X}$, geometric mean; R, range. Sample size for all variables, N = 7.

		Chinook salmon test[a]						Striped bass test[a]					
Variable		WWD	RWWD	IO	SJR	RSJR	MR	WWD	RWWD	IO	DELTA	RDELTA	EGH
Ammonia (mg/L)	$\bar{X}$	1.60	1.44	3.76	0.492	0.277	0.0361	0.581	0.0514	1.06	0.108	0.0659	0.0928
	R	1.3-2.2	1.1-2.3	3.4-4.8	0.44-0.74	0.12-0.88	0.010-0.077	0.000-4.8	0.000-0.34	0.050-5.1	0.0025-0.38	0.000-0.22	0.000-0.43
Chloride (mg/L)	$\bar{X}$	1,240	978	7,330	253	239	2.29	1,040	947	5,540	89.1	79.2	11.1
	R	1,100-1,400	870-1,100	7,200-7,600	190-310	230-250	2.0-2.7	970-1,100	930-970	4,800-6,200	67-120	52-120	9.5-12.0
Hardness (mg/L as $CaCO_3$)													
Calcium	$\bar{X}$	1,140	721	603	218	215	6.28	1,090	930	542	61.6	62.2	61.8
	R	1,100-1,200	610-920	530-660	180-250	170-240	6.1-6.5	1,100-1,100	880-990	480-610	46-86	39-95	56-67
Magnesium	$\bar{X}$	746	494	2,180	163	149	2.47	536	518	1,770	57.5	59.0	63.9
	R	660-830	400-670	2,000-2,300	110-200	130-170	2.1-2.8	510-570	440-570	1,600-1,900	46-80	35-80	57-68
Total	$\bar{X}$	1,890	1,220	2,780	381	364	8.76	1,630	1,450	2,320	119	121	126
	R	1,800-2,000	1,000-1,600	2,500-2,900	300-450	300-410	8.6-9.0	1,600-1,600	1,400-1,500	2,200-2,400	96-160	74-180	120-130
Sodium (mg/L)	$\bar{X}$	5,780	4,140	4,290	236	230	1.57	4,400	4,380	3,210	56.2	49.6	18.3
	R	4,700-7,300	3,100-5,900	3,800-5,200	170-280	210-270	1.3-1.8	3,600-4,900	3,900-4,800	2,900-3,500	39-84	27-74	17-19
Sulfate (mg/L)	$\bar{X}$	13,300	9,170	1,120	316	391	0.00	9,200	8,860	846	41.3	42.8	0.00
	R	11,000-17,000	7,000-14,000	900-1,300	230-370	350-490	0.00-0.00	7,400-10,000	8,300-9,600	750-900	28-72	20-64	0.00-0.00
Total alkalinity													
(mg/L as $CaCO_3$)	$\bar{X}$	187	131	276	176	137	14.9	166	118	289	80.7	73.0	146
	R	180-190	120-160	230-310	150-220	130-150	5.7-18	150-170	120-120	230-370	73-94	43-110	150-150

Table 2. (Continued)

Variable		Chinook salmon test[a]						Striped bass test[a]					
		WWD	RWWD	IO	SJR	RSJR	MR	WWD	RWWD	IO	DELTA	RDELTA	EGH
Total dissolved solids													
(mg/L)	$\bar{X}$	20,500	14,300	16,000	1,160	1,280	28.3	15,600	14,700	12,100	315	280	221
	R	18,000-23,000	12,000-18,000	16,000-16,000	900-1,400	1,000-2,500	10-50	14,000-17,000	15,000-15,000	12,000-13,000	250-450	140-390	180-260
Turbidity (NTU's)	$\bar{X}$	0.405	0.192	0.352	14.5	0.180	0.355	0.235	0.369	0.405	47.2	0.0815	0.171
	R	0.21-0.74	0.045-0.30	0.25-0.51	4.5-52	0.020-0.30	0.22-0.46	0.12-0.33	0.11-0.65	0.15-0.62	32-62	0.010-0.16	0.010-0.38

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[a] Water types were as follows: WWD, tile drainage water from Sump #20 in the Westlands Water District; RWWD, reconstituted drainage water; IO, reconstituted seawater; SJR, water from the San Joaquin River at Crows Landing Road; RSJR, reconstituted river water; MR, water from the Merced River Fish Facility; DELTA, water from the Sacramento-San Joaquin Delta at the Tracy Fish Screen; RDELTA, reconstituted delta water; and EGH, water from the Central Valley's Fish Hatchery.

Table 3. Dissolved concentrations (µg/L) of boron, chromium, copper, molybdenum, and selenium in full-strength tile drainwater (WWD) and other water types (IO, reconstituted seawater [Instant Ocean^R]; MR, water from the Merced River Fish Facility; RSJR, reconstituted river water; SJR, water from the San Joaquin River; RWWD, reconstituted drainage water; WWD, water from the Westlands Water District [tile drainage water]; EGH, water from the Central Valley's Fish Hatchery; RDELTA, reconstituted delta water; and DELTA, water from the Sacramento-San Joaquin Delta). $\bar{X}$, geometric mean; N, number of samples; and R, range.

Test	Water type		Boron	Chromium	Copper	Molbdenum	Selenium
Chinook salmon	Blank	$\bar{X}$ (N)	<200. (3)	0.758 (21)	<2.50 (21)	<50.0 (3)	<0.600 (21)
		R	<200.-<200.	<0.80-2.8	<2.5-<2.5	<50.-<50.	<0.60-<0.60
	IO	$\bar{X}$ (N)	2,650. (3)	19.5 (21)	13.2 (21)	<50.0 (3)	1.13 (21)
		R	2,400-2,800.	9.7-26.	11.-18.	<50.-<50.	<0.40-2.2
	MR	$\bar{X}$ (N)	<200. (3)	0.498 (21)	1.49 (21)	<50.0 (3)	0.244 (21)
		R	<200.-<200.	<0.90-1.4	<2.5-3.8	<50.-<50.	<0.40-0.40
	RSJR	$\bar{X}$ (N)	<200. (3)	0.606 (21)	0.857 (21)	<50.0 (3)	<1.29 (21)
		R	<200.-<200.	<0.80-2.8	<1.2-2.1	<50.-<50.	<0.60-<1.4
	SJR	$\bar{X}$ (N)	1,170. (3)	0.692 (21)	2.00 (21)	<50.0 (3)	6.45 (21)
		R	900.-1,600.	<0.90-6.8	<2.5-4.0	<50.-<50.	4.8-8.1
	RWWD	$\bar{X}$ (N)	<200. (3)	0.971 (21)	0.623 (21)	<50.0 (3)	0.347 (21)
		R	<200.-<200.	<0.80-2.4	<1.2-1.3	<50.-<50.	<0.40-0.90
	WWD	$\bar{X}$ (N)	48,800. (3)	25.4 (21)	4.02 (21)	766. (3)	218. (21)
		R	44,000.-53,000.	15.-36.	2.5-7.5	650.-910.	54.-290.
Striped bass	Blank	$\bar{X}$ (N)	<200. (3)	0.864 (21)	1.55 (21)	<50.0 (3)	<0.600 (21)
		R	<200.-<200.	1.6-2.0	<2.5-4.7	<50.-<50.	<0.60-<0.60
	IO	$\bar{X}$ (N)	2,340. (3)	17.8 (21)	8.81 (21)	31.5 (3)	1.06 (21)
		R	2,100.-2,600	5.3-32.	7.5-12.	<50.-50.	<0.50-2.6
	EGH	$\bar{X}$ (N)	<200. (3)	5.08 (21)	<2.80 (21)	<50.0 (3)	<0.422 (21)
		R	<200.-<200.	3.4-6.9	<2.8-<2.8	<50.-<50.	<0.30-<0.50
	RDELTA	$\bar{X}$ (N)	192. (3)	0.414 (21)	<2.30 (21)	46.4 (3)	<0.373 (21)
		R	<200.-710.	<0.60-1.4	<2.3-<2.3	<50.-80.	<0.30-<0.50
	DELTA	$\bar{X}$ (N)	139. (3)	0.608 (21)	1.71 (21)	31.5 (3)	0.532 (21)
		R	<200.-270.	<0.60-2.5	<2.8-4.1	<50.-50.	<0.40-1.3

Table 3. (Continued)

Test	Water type		Boron	Chromium	Copper	Molbdenum	Selenium
Striped bass (Continued)							
	RWWD	$\bar{X}$ (N)	<200. (3)	<1.50 (21)	<2.30 (21)	<50.0 (3)	0.258 (21)
		R	<200.-<200.	<1.5-<1.5	<2.3-<2.3	<50.-<50.	<0.50-0.50
	WWD	$\bar{X}$ (N)	49,300. (3)	24.0 (21)	4.32 (21)	663. (3)	158. (21)
		R	45,000-52,000	17.-34.	3.3-6.5	590.-760.	130.-270.

Table 4. Boron, molybdenum, and selenium concentrations (µg/g dry weight) in whole chinook salmon and striped bass held for up to 28 days in serial dilutions of tile drainage water (WWD) and full-strength concentrations of other treatments (MR, water from the Merced River Fish Facility; IO, reconstituted seawater [Instant Ocean^{R}]; RWWD, reconstituted drainage water; SJR, dilution water from the San Joaquin River; EGH, water from the Central Valley's Fish Hatchery; and DELTA, dilution water from the Sacramento-San Joaquin Delta). $\bar{X}$, geometric mean; N, number of samples; and R, range.

Species	Treatment		Boron	Molybdenum	Selenium
Chinook salmon	MR	$\bar{X}$ (N)	1.21 (6)	<0.295 (6)	1.32 (6)
		R	<1.6-4.9	<0.30-<0.30	1.2-1.4
	IO	$\bar{X}$ (N)	2.10 (6)	<0.318 (6)	1.40 (6)
		R	<1.6 - 5.8	<0.30-<0.33	1.3-1.5
	RWWD	$\bar{X}$ (N)	<1.59 (6)	0.167 (6)	1.39 (6)
		R	<1.6-<1.6	<0.30-0.31	1.3-1.5
	SJR	$\bar{X}$ (N)	<1.59 (6)	0.169 (6)	1.51 (6)
		R	<1.6-<1.6	<0.30-0.33	1.4-1.7
	WWD12.5	$\bar{X}$ (N)	10.3 (6)	<0.295 (6)	1.39 (6)
		R	6.4-16.	<0.30-<0.30	1.3-1.4
	WWD25	$\bar{X}$ (N)	12.4 (6)	0.210 (6)	1.50 (6)
		R	9.6-19.	<0.30-0.59	1.4-1.7
	WWD50	$\bar{X}$ (N)	19.3 (6)	0.171 (6)	1.92 (6)
		R	14.-27.	<0.30-0.36	1.5-5.7
	WWD100	$\bar{X}$ (N)	228. (8)	0.404 (8)	2.12 (9)
		R	190.-320.	<0.30-1.0	2.0-2.4
Striped bass	EGH	$\bar{X}$ (N)	<3.06 (6)	0.220 (6)	1.23 (7)
		R	<3.1-<3.1	<0.33-0.43	1.1-1.4
	IO	$\bar{X}$ (N)	2.15 (6)	<0.309 (6)	1.06 (6)
		R	<3.8-4.0	<0.31-<0.31	0.43-1.4
	RWWD	$\bar{X}$ (N)	<3.79 (6)	01.80 (6)	1.22 (7)
		R	<3.8-<3.8	<0.31-0.39	1.1-1.5

Table 4. (Continued)

Species	Treatment		Boron	Molbdenum	Selenium
Striped bass (Continued)					
	DELTA	$\bar{X}$ (N)	<3.06 (6)	0.191 (6)	1.21 (7)
		R	<3.1-<3.1	<0.33-0.40	0.99-1.4
	WWD12.5	$\bar{X}$ (N)	2.25 (6)	<0.309 (6)	1.37 (6)
		R	<3.8-5.3	<0.31-<0.31	1.2-1.5
	WWD25	$\bar{X}$ (N)	2.00 (6)	<0.326 (6)	1.38 (7)
		R	<3.1-7.8	<0.31-<0.33	1.3-1.6
	WWD50	$\bar{X}$ (N)	9.85 (8)	0.210 (8)	1.47 (9)
		R	4.3-49.	<0.31-0.72	1.3-3.0
	WWD100	$\bar{X}$ (N)	136. (6)	0.223 (6)	1.26 (6)
		R	90.-190.	<0.31-0.48	1.1-1.4

Table 5. Pearson product-moment correlations (*r*) between toxic endpoints (survival, growth in total length and weight, and relative condition factor) of chinook salmon and striped bass and selected physico-chemical water quality variables.[a]

Water quality	Chinook salmon				Striped bass			
variables	Survival	TL[b]	Wt[c]	Kn[d]	Survival	TL[b]	Wt[c]	Kn[d]
Physical								
Conductivity	-0.27	-0.32	-0.32	-0.21	-0.50	-0.28	-0.42	-0.59*
Temperature	-0.80**	-0.72**	-0.77**	-0.80**	-0.86**	-0.58*	-0.66*	-0.69**
Turbidity	0.35	0.50	0.45	0.03	0.47	0.57*	0.48	0.13
Chemical								
Boron	-0.50	-0.42	-0.43	-0.42	-0.27	0.13	0.18	0.24
Chloride	-0.13	-0.12	-0.13	-0.13	-0.18	-0.19	-0.27	-0.34
Chromium	-0.49	-0.50	-0.48	-0.28	-0.20	-0.09	0.03	0.32
Copper	-0.15	-0.07	-0.06	-0.06	-0.02	-0.07	0.02	0.25
Dissolved oxygen	-0.47	-0.34	-0.37	-0.51	0.07	0.24	0.30	0.37
Molybdenum	-0.65**	-0.67**	-0.66**	-0.44	-0.47	0.10	0.14	0.17
Sodium	-0.27	-0.31	-0.32	-0.23	-0.47	-0.21	-0.36	-0.59*
Ammonia	-0.21	-0.26	-0.24	-0.05	<0.01	-0.07	0.01	0.24
pH	-0.32	-0.25	-0.28	-0.35	-0.40	-0.31	-0.27	-0.09
Selenium	-0.56*	-0.50	-0.52*	-0.50	-0.42	0.16	0.17	0.13
Sulfate	-0.31	-0.36	-0.37	-0.28	-0.50	-0.08	-0.27	-0.62*
Total dissolved solids	-0.29	-0.34	-0.34	-0.23	-0.50	-0.28	-0.41	-0.60*
Total alkalinity	-0.09	-0.02	-0.04	-0.17	-0.16	-0.35	-0.28	-0.02
Total hardness	-0.23	-0.25	-0.25	-0.21	-0.47	-0.39	-0.48	-0.51

[a] Codes: *, *P* ≤0.05; **, *P* ≤0.01.

[b] Total length.

[c] Weight.

[d] Relative condition factor.

Figure 1. Survival of chinook salmon fingerlings exposed for 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (r values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the San Joaquin River; MR, supplemental control from the Merced River Fish Facility (hatchery); RSJR, supplemental control composed of reconstituted river water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each r value indicates a significant correlation ($P \leq 0.05$, based on Student's two-tailed t test; "*" above each bar indicates a significant difference from the CONTROL ($P \leq 0.05$, based on Dunnett's one-tailed t test).

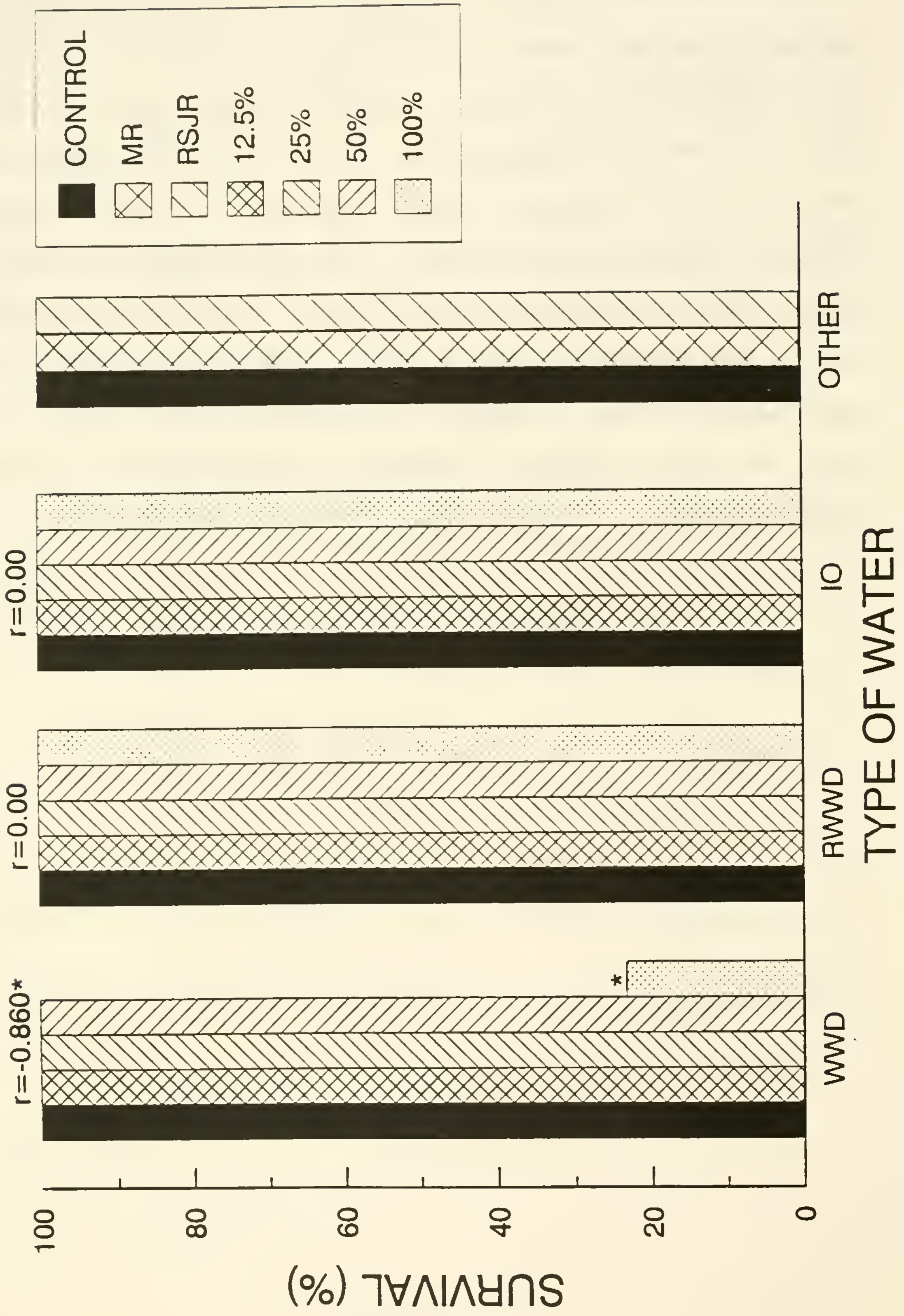

CONTROL
MR
RSJR
12.5%
25%
50%
100%
r=-0.860*
r=0.00
r=0.00
*
WWD
RWWD
IO
OTHER
TYPE OF WATER
SURVIVAL (%)
100
80
60
40
20
0

Figure 2. Total lengths attained by chinook salmon fingerlings exposed for up to 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (r values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the San Joaquin River; MR, supplemental control from the Merced River Fish Facility (hatchery); RSJR, supplemental control composed of reconstituted river water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each r value indicates a significant correlation ($P \le 0.05$, based on Student's two-tailed t test; "*" above each bar indicates a significant difference from the CONTROL ($P \le 0.05$, based on Dunnett's one-tailed t test).

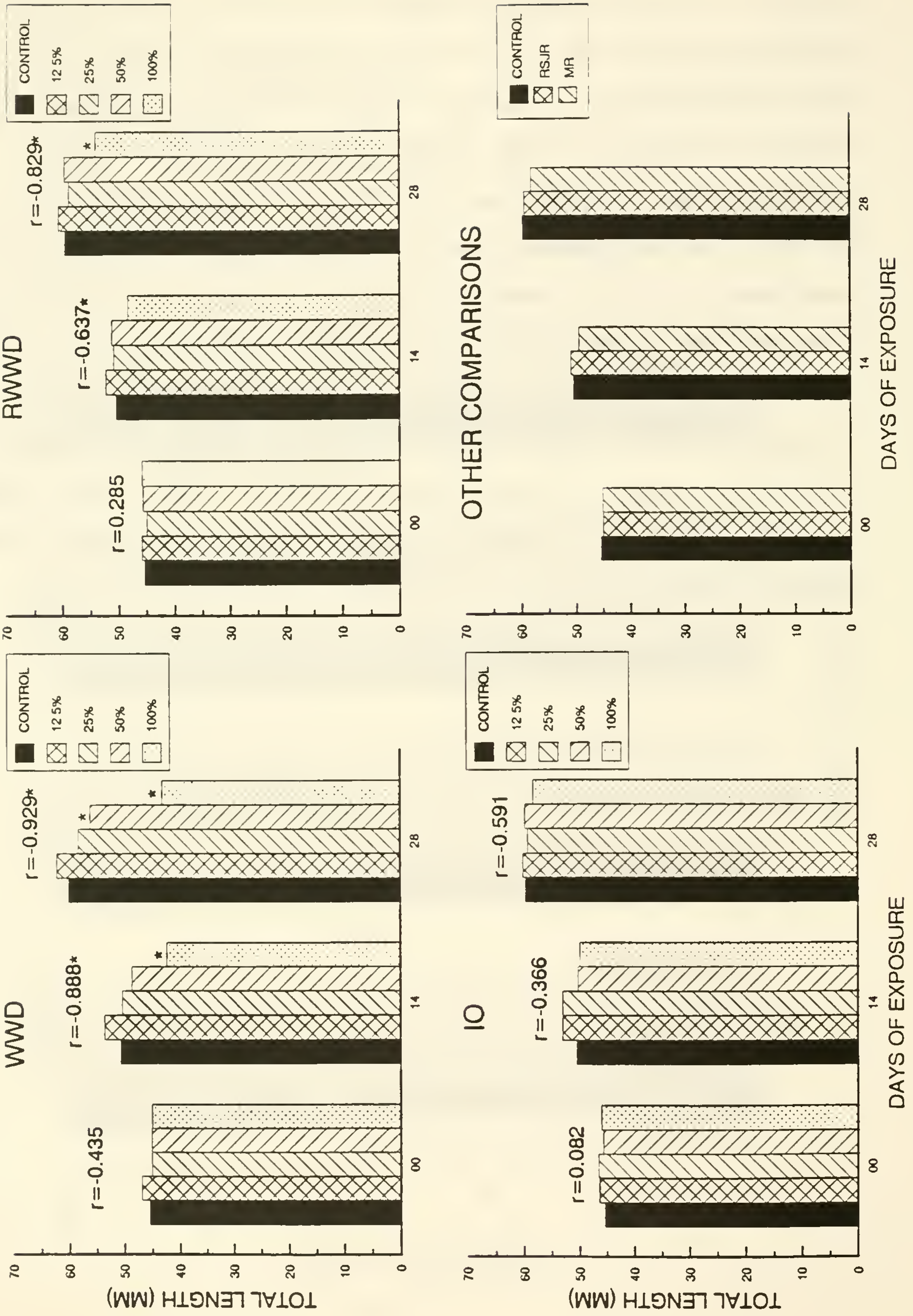

WWD
r=-0.435
r=-0.888*
r=-0.929*
*
CONTROL
12 5%
25%
50%
100%
RWWD
r=0.285
r=-0.637*
r=-0.829*
*
CONTROL
12 5%
25%
50%
100%
IO
r=0.082
r=-0.366
r=-0.591
CONTROL
12 5%
25%
50%
100%
OTHER COMPARISONS
CONTROL
RSJR
MR
TOTAL LENGTH (MM)
0
10
20
30
40
50
60
70
00
14
28
DAYS OF EXPOSURE

Figure 3. Weights attained by chinook salmon fingerlings exposed for up to 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (r values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the San Joaquin River; MR, supplemental control from the Merced River Fish Facility (hatchery); RSJR, supplemental control composed of reconstituted river water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each r value indicates a significant correlation ($P \le 0.05$, based on Student's two-tailed t test; "*" above each bar indicates a significant difference from the CONTROL ($P \le 0.05$, based on Dunnett's one-tailed t test).

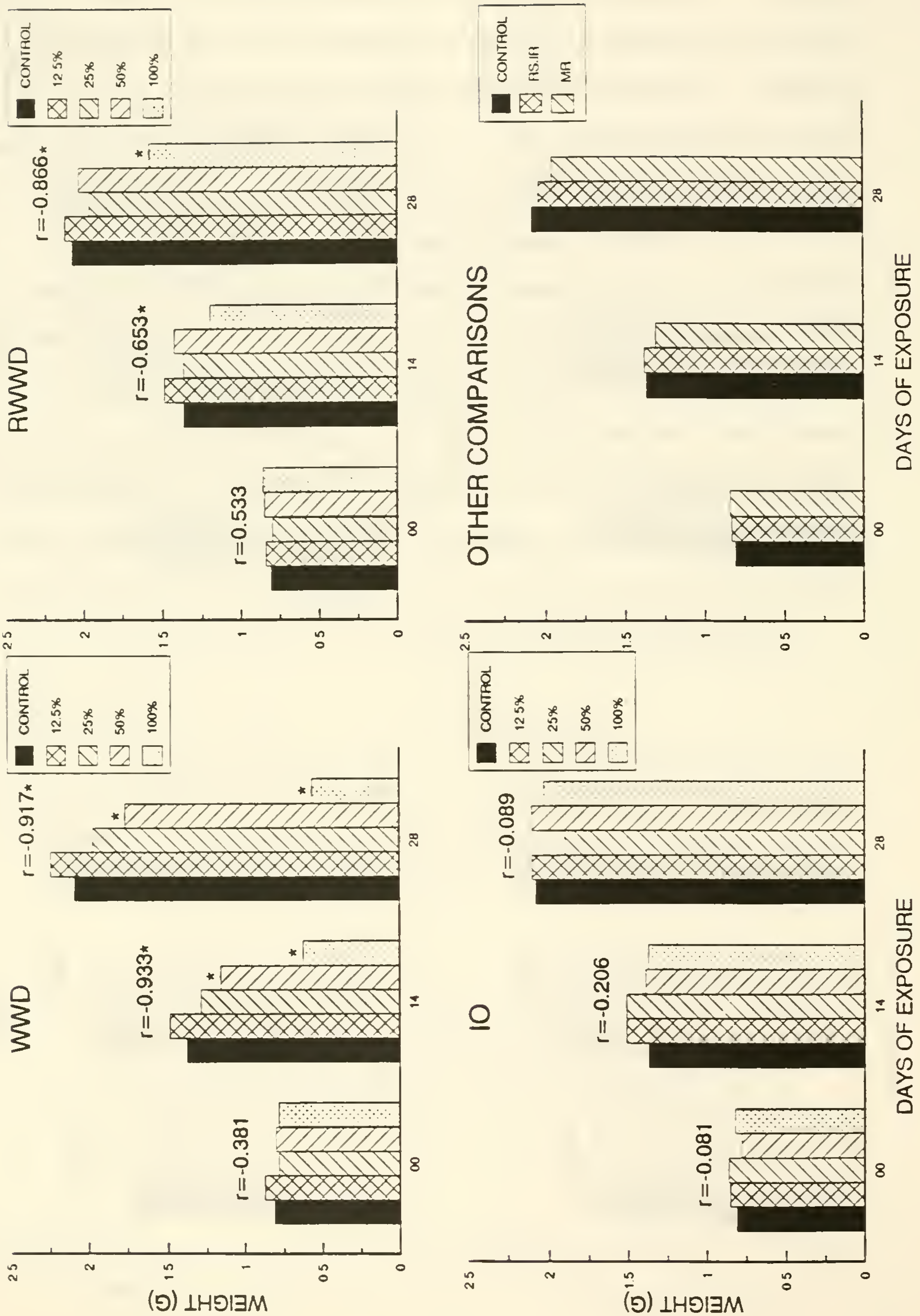

WWD
r=-0.381
r=-0.933*
r=-0.917*
*
CONTROL
12.5%
25%
50%
100%
RWWD
r=0.533
r=-0.653*
r=-0.866*
IO
r=-0.081
r=-0.206
r=-0.089
OTHER COMPARISONS
RS.IR
MR
WEIGHT (G)
0
0.5
1
1.5
2
2.5
00
14
28
DAYS OF EXPOSURE

Figure 4. Relative condition factors of chinook salmon fingerlings exposed for up to 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (r values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the San Joaquin River; MR, supplemental control from the Merced River Fish Facility (hatchery); RSJR, supplemental control composed of reconstituted river water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each r value indicates a significant correlation ($P \leq 0.05$, based on Student's two-tailed t test; "*" above each bar indicates a significant difference from the CONTROL ($P \leq 0.05$, based on Dunnett's one-tailed t test).

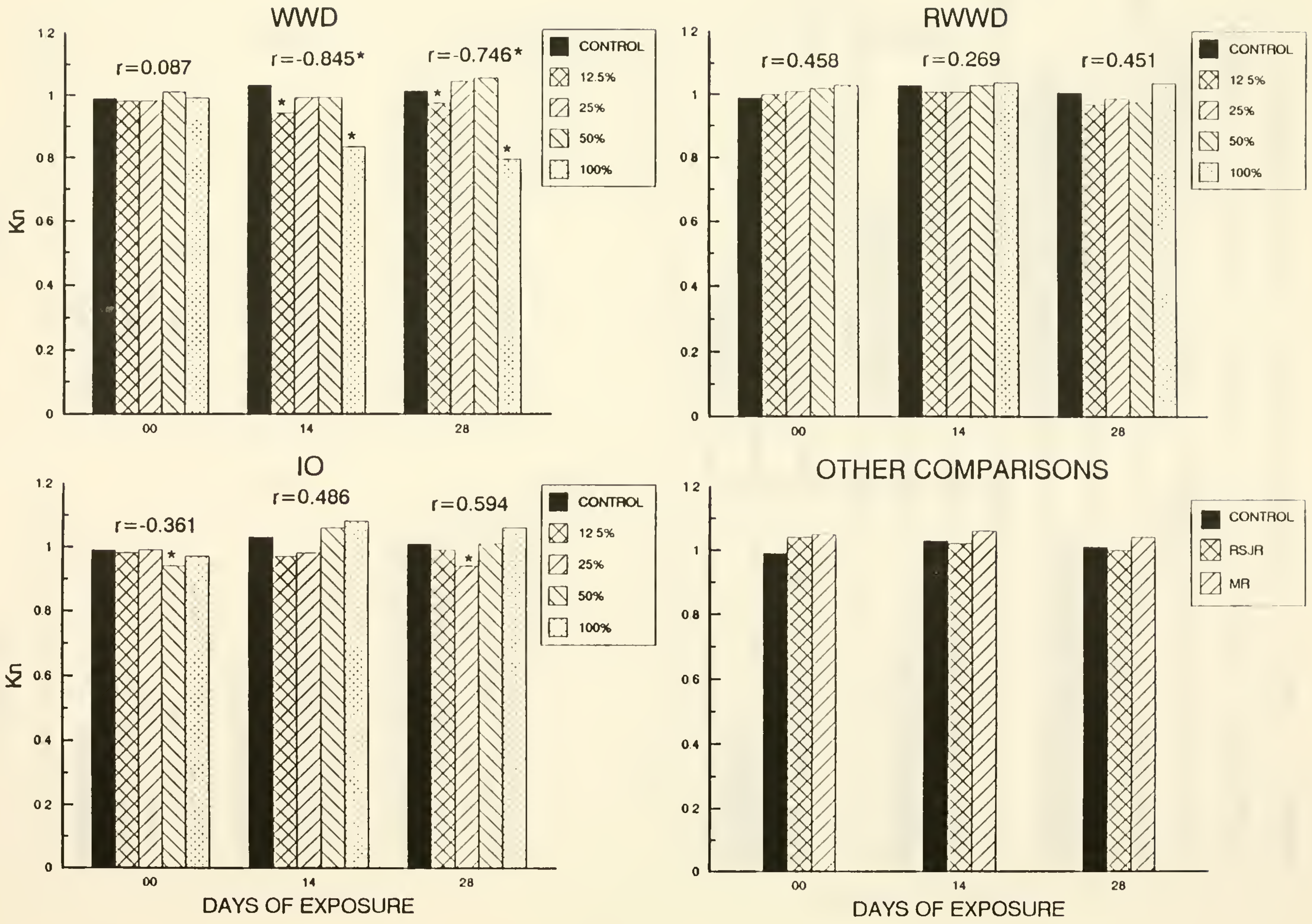

WWD
r=0.087
r=-0.845*
r=-0.746*
*
Kn
1.2
1
0.8
0.6
0.4
0.2
0
00
14
28
CONTROL
12.5%
25%
50%
100%
RWWD
r=0.458
r=0.269
r=0.451
IO
r=-0.361
r=0.486
r=0.594
DAYS OF EXPOSURE
OTHER COMPARISONS
RSJR
MR

Figure 5. Survival of striped bass fingerlings exposed for 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (*r* values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; EGH, supplemental control from the Central Valleys Fish Hatchery; RDELTA, supplemental control composed of reconstituted delta water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each *r* value indicates a significant correlation ($P \leq 0.05$, based on Student's two-tailed *t* test; "*" above each bar indicates a significant difference from the CONTROL ($P \leq 0.05$, based on Dunnett's one-tailed *t* test).

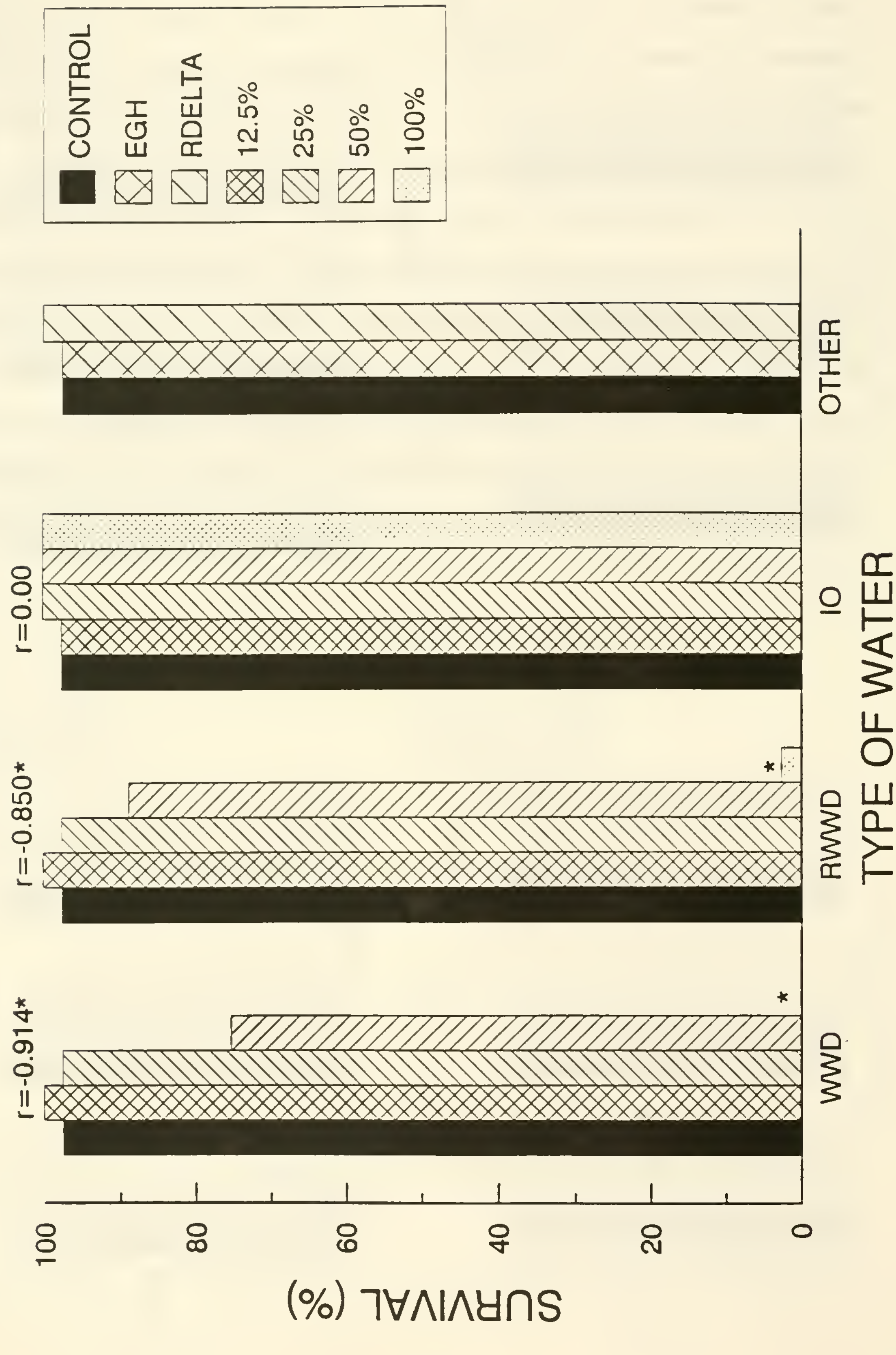

SURVIVAL (%)
100
80
60
40
20
0
r=-0.914*
r=-0.850*
r=0.00
*
*
WWD
RWWD
IO
OTHER
TYPE OF WATER
CONTROL
EGH
RDELTA
12.5%
25%
50%
100%

Figure 6. Total lengths attained by striped bass fingerlings exposed for up to 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (r values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; EGH, supplemental control from the Central Valleys Fish Hatchery; RDELTA, supplemental control composed of reconstituted delta water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each r value indicates a significant correlation ($P \leq 0.05$, based on Student's two-tailed t test; "*" above each bar indicates a significant difference from the CONTROL ($P \leq 0.05$, based on Dunnett's one-tailed t test).

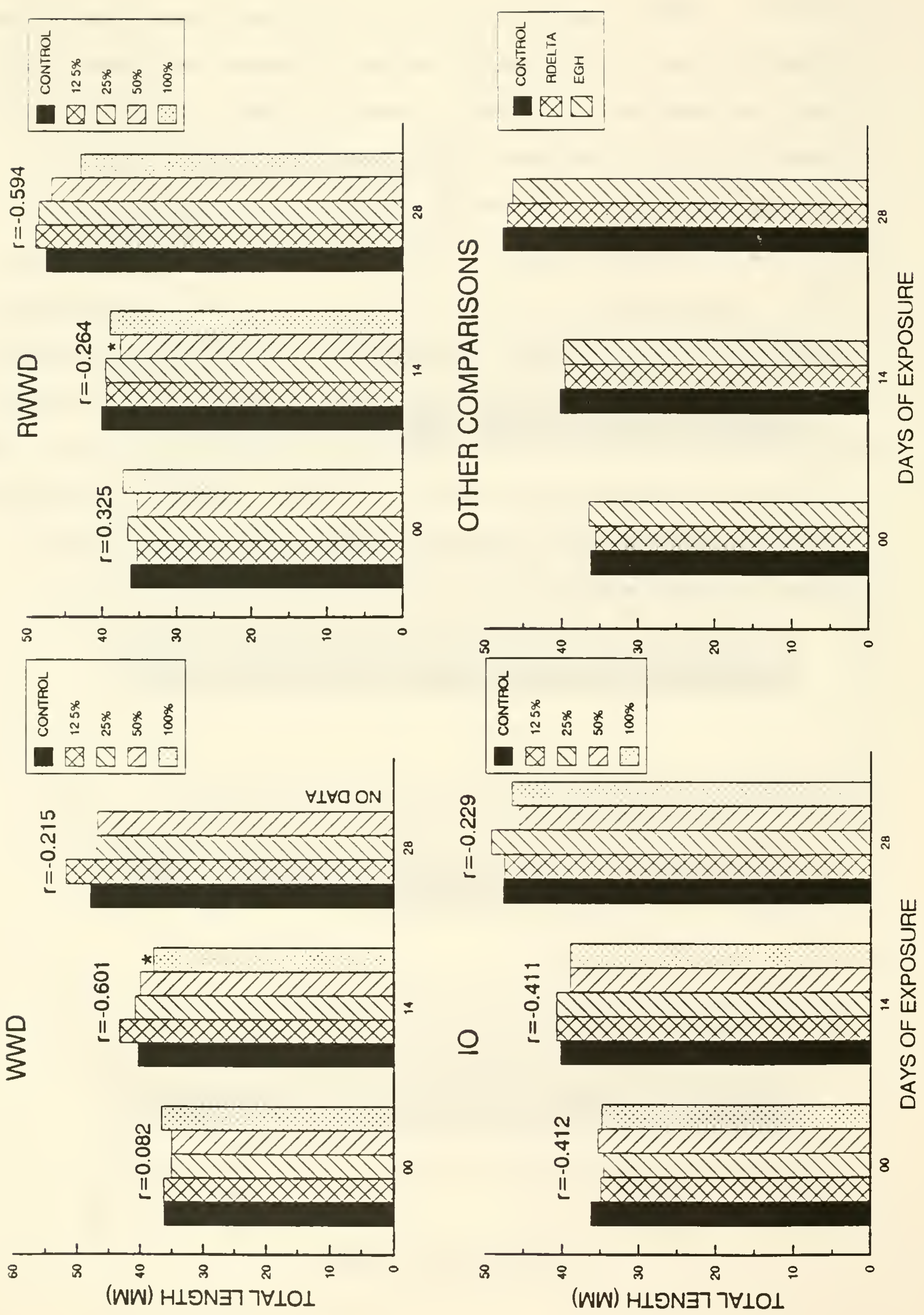
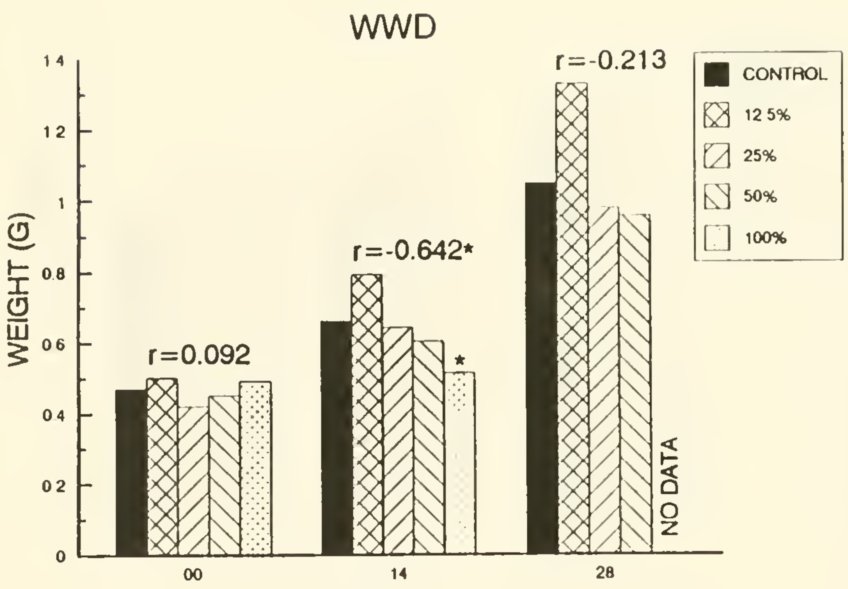
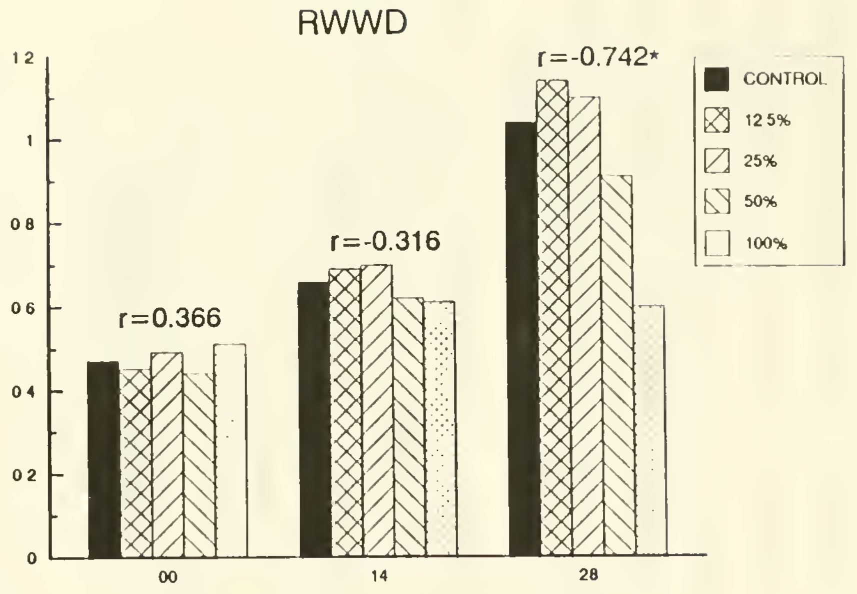
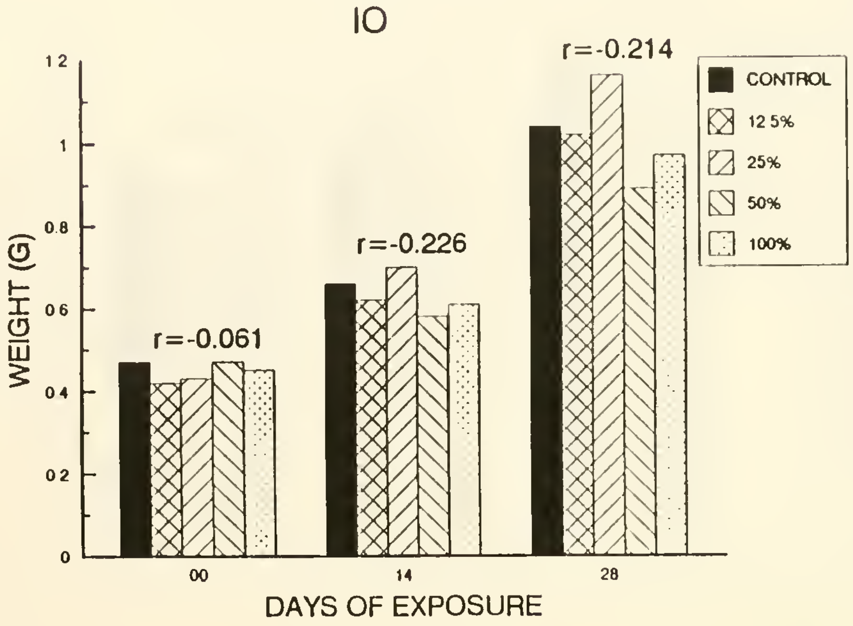
WWD
RWWD
IO
OTHER COMPARISONS
TOTAL LENGTH (MM)
DAYS OF EXPOSURE
00
14
28
0
10
20
30
40
50
60
r=0.082
r=-0.601
r=-0.215
r=0.325
r=-0.264
r=-0.594
r=-0.412
r=-0.411
r=-0.229
*
NO DATA
CONTROL
12.5%
25%
50%
100%
RDELTA
EGH

Figure 7. Weights attained by striped bass fingerlings exposed for up to 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (*r* values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; EGH, supplemental control from the Central Valleys Fish Hatchery; RDELTA, supplemental control composed of reconstituted delta water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each *r* value indicates a significant correlation ($P \leq 0.05$, based on Student's two-tailed *t* test; "*" above each bar indicates a significant difference from the CONTROL ($P \leq 0.05$, based on Dunnett's one-tailed *t* test).


WWD
WEIGHT (G)
0
0 2
0 4
0 6
0.8
1
1 2
1 4
00
14
28
r=0.092
r=-0.642*
*
r=-0.213
NO DATA
CONTROL
12 5%
25%
50%
100%


RWWD
0
0 2
0 4
0 6
0 8
1
1 2
00
14
28
r=0.366
r=-0.316
r=-0.742*
CONTROL
12 5%
25%
50%
100%


IO
WEIGHT (G)
0
0 2
0 4
0 6
0.8
1
1 2
00
14
28
DAYS OF EXPOSURE
r=-0.061
r=-0.226
r=-0.214
CONTROL
12 5%
25%
50%
100%

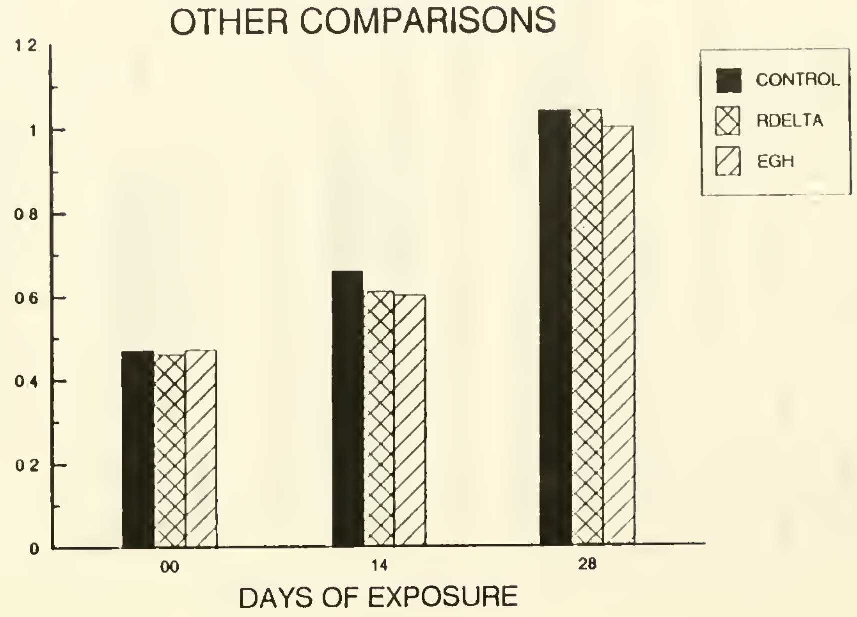
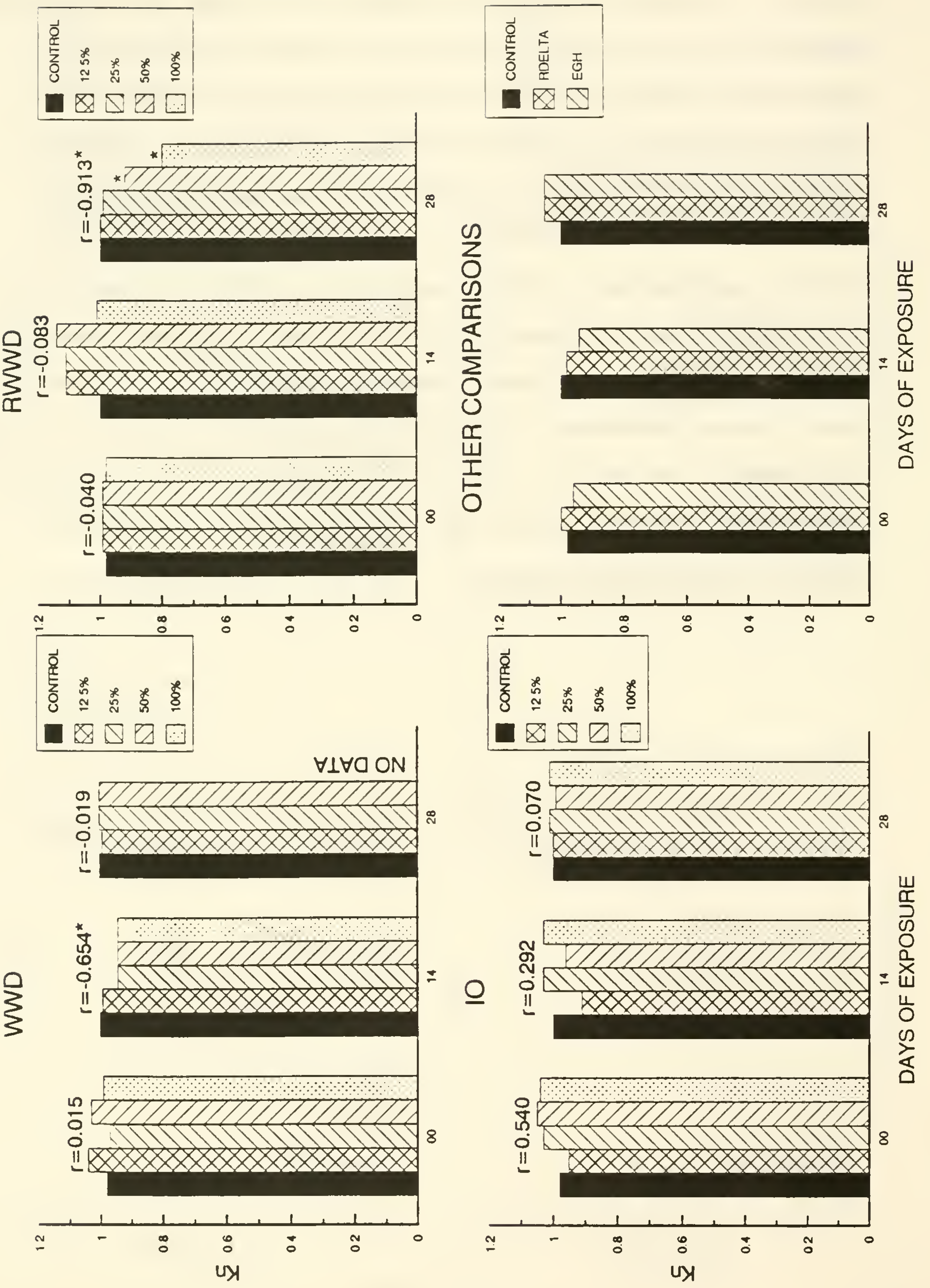

OTHER COMPARISONS
0
0 2
0 4
0 6
0 8
1
1 2
00
14
28
DAYS OF EXPOSURE
CONTROL
RDELTA
EGH

Figure 8. Relative condition factors of striped bass fingerlings exposed for up to 28 days to serial dilutions of three water types and controls. Pearson's product-moment correlations (*r* values) are given for WWD, RWWD, and IO. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; EGH, supplemental control from the Central Valleys Fish Hatchery; RDELTA, supplemental control composed of reconstituted delta water; WWD, tile drainage water; RWWD, reconstituted drainage water; and IO, reconstituted seawater. Codes: "*" following each *r* value indicates a significant correlation ($P \leq 0.05$, based on Student's two-tailed *t* test; "*" above each bar indicates a significant difference from the CONTROL ($P \leq 0.05$, based on Dunnett's one-tailed *t* test).


WWD
r=0.015
r=-0.654*
r=-0.019
NO DATA
RWWD
r=-0.040
r=-0.083
r=-0.913*
*
*
IO
r=0.540
r=0.292
r=0.070
OTHER COMPARISONS
Kn
Kn
0
0.2
0.4
0.6
0.8
1
1.2
00
14
28
DAYS OF EXPOSURE
DAYS OF EXPOSURE
CONTROL
12.5%
25%
50%
100%
CONTROL
RDELTA
EGH

EFFECTS OF SELENIUM ON BLUEGILL REPRODUCTION

Introduction

Due to the seleniferous nature of soil on the west side of the San Joaquin Valley, California, potentially toxic selenium (Se) concentrations may accumulate in irrigation drainage water. Selenium concentrations up to 1350 μg/L have been measured in certain evaporative basins in the region (Presser and Barnes, 1984). These waterborne Se concentrations greatly exceed worldwide ambient concentrations in rivers (0.2 μg/L) and seas (0.09 μg/L; Presser and Barnes, 1984). Concerns developed regarding the potential threat that elevated Se in irrigation drainage water may pose to fish and wildlife inhabiting the region. A specific concern was the potential effects of Se on the reproductive success of striped bass and chinook salmon -- two economically important anadromous species. To address this concern, the National Fisheries Contaminant Research Center, through an intra-agency agreement with the U.S. Bureau of Reclamation, conducted a series of acute and chronic toxicity studies to evaluate the relative toxicity of various forms of Se to fish and assess potential effects on reproduction. Because of the complexity of the life cycle of anadromous fishes, a surrogate species, bluegill sunfish, was selected for the studies. These studies included 1) a series of acute exposures of juvenile bluegill to various forms of waterborne Se, 2) chronic exposures of juvenile bluegill to waterborne and dietary Se, and 3) a chronic dietary exposure of adult bluegill to organoselenium and subsequent evaluation of reproductive success.

Methods

1) Acute waterborne Se toxicity studies: A series of acute waterborne toxicity studies

with bluegill were conducted in both a generic freshwater (described later) and standard ASTM soft water (hardness, 40-48 mg/L as CaCO3; alkalinity, 30-35 mg/L as CaCO3; pH, 7.2-7.6). Juvenile bluegill were exposed under static conditions for 96-hours to waterborne sodium selenate, sodium selenite, a 6:1 mixture of sodium selenate to sodium selenite, seleno-L-methionine, and seleno-DL-methionine. The studies were conducted in 19.6 L glass jars containing 15 L of exposure water. Treatments were spiked to obtain the desired exposure concentrations and there was a 60% dilution factor between each concentration. Ten fish were placed into each jar and mortality was monitored every 24 hours. Water samples were taken from each treatment at 0 and 96-hours of the studies and analyzed for total Se. In addition, filtered samples were taken from the control, low, medium, and high concentrations of the mixture studies and analyzed for selenate and selenite.

2) Chronic waterborne and dietary Se toxicity studies: Prior to initiating the bluegill reproductive study, three range-finding partial chronic toxicity studies were conducted in modified versions of the flow-through proportional diluter of Mount and Brungs (1967). The juvenile bluegill used in the studies were obtained from Mammoth Springs National Fish Hatchery, Mammoth Springs, AR, and held in well water (pH 7.8, alkalinity 255 and hardness 283 mg/L as $CaCO_3$) at 17°C prior to starting the studies. Exposure water for the studies was reconstituted according to the precedures of Ingersoll et al. (1990). Appropriate amounts of well water, reverse osmosis (RO) water, calcium sulfate, and magnesium sulfate were blended in 8327 liter polyolefin tanks to yield a final exposure water that contained 28 mg/L calcium, 14 mg/L potassium, 5 mg/L chlorides, 72 mg/L sulfates, 60-65 mg/L alkalinity as $CaCO_3$, 134 mg/L hardness as $CaCO_3$, and a pH of about 8.1. This water was representative of surface waters in the San Joaquin Valley. Before initiating the study the fish were acclimated from well water to exposure water quality over a 5-day period.

In the first study (Exposure A), young bluegill about 3 months old and weighing 0.2 g were exposed to a 6:1 waterborne mixture of sodium selenate to sodium selenite (Sigma Chemical Company, St. Louis, Missouri) for 29 days. Nominal exposure concentrations of total Se were 21.7, 10.9, 5.4, 2.7, and 1.4 mg/L. Sodium selenate (98% pure, 41.8% Se) and sodium selenite (98% pure, 45% Se) stock solutions were prepared in RO water at concentrations that were corrected for % purity and % Se in the compounds. The stock solutions were dispensed to the diluter with automatic pipettes to maintain the exposure concentrations. The study was conducted at 25°C and the fish were fed a commercial diet 3 times daily. Fish were also exposed to duplicate controls with no Se added. The exposure containers were 79 X 32 X 30.5 cm (length X width X height) glass aquaria equipped with 20.5 cm high standpipes. The exposure volume was 51.8 L, and the aquaria were divided into two 8.7 L chambers and one 34.1 L chamber.

Ten bluegill were placed into each small chamber of the aquaria for growth determinations and 40 fish were placed in each of the larger chambers for behavior and tissue residue measurements. Growth samples were taken at 0, 15, and 29 days of exposure, and samples for tissue analysis for Se were taken at 29 days. Mortality in all treatments was recorded daily.

Behavioral responses of bluegill were assessed by behavioral surveys and swimming behavior tests. Beginning after the first week of exposure, behavioral surveys modified from Drummond et al. (1986) and Mehrle et al. (1988) were conducted several times each week to characterize abnormal responses. The responses included changes in coloration, activity, excitability by external stimuli, location in aquaria, mode of swimming, feeding, and aggressive interactions. Observations were made by the same observer each day prior to the noon or afternoon feedings. A behavior was judged aberrant when fish of a given treatment swam or reacted in a manner that obviously differed from that of controls. The large number of fish stocked in each exposure chamber precluded an accurate count of affected individuals; therefore, a behavioral change was noted if 10% of an exposure tank population exhibited the responses. Although no attempt was made to quantify the number of fish responding abnormally, an overall measure of the onset and sequence of behavioral changes was made from the systematic daily observations. The repeated observation of abnormal responses within a treatment group over time was used as criterion for determining behavioral changes. Behavioral changes were assessed by plotting the first day of exposure on which fish from both replications of a Se treatment showed abnormal behavior. Because of the all-or-none nature of the survey observations, further statistical analyses were not applied.

Behavioral impairment of bluegill was further quantified by measuring locomotory activity of fish that survived three weeks of the exposures. Ten fish from each exposure chamber were confined in a 15x13 cm area by placing a glass partition in the exposure chamber. After a 20-minute acclimation period the fish were video taped for five minutes with an overhead video camera. Duration of movement was measured on individual fish with a stopwatch as cumulative time of continuous movement from minute 2 to minute 3 of the recording. The frequency of swimming bouts was measured over this same period of time as frequency of activity. The speed of movement and distance moved by each fish was also measured during the first 15 seconds of this period using an IBM PC-based video processing system (Motion Analysis Corp, Santa Rosa, CA) following the procedures described by Smith and Bailey (1988).

The temperature was monitored daily, and pH, hardness, alkalinity, conductivity (APHA et al., 1975), chlorides (Orion Research, Inc., 1984), and sulfates (Hulanicki et al.

1976) of the exposure water were determined once weekly. The pH, alkalinity, hardness, and sulfate were also measured on each tank of experimental water. Two-hundred ml of exposure water were collected from mid-water column of each treatment and prepared for Se analysis at day 0 and every two weeks thereafter. Four-hundred ml of exposure water were collected from the low, medium, and high concentrations, filtered, and acidified for Se speciation analysis at day 0 and 29.

Se concentrations in Exposure A were too high to determine no-effect Se concentrations. Thus, the second study (Exposure B) was conducted in which 5-month-old bluegill weighing about 0.3 g were exposed to lower waterborne Se concentrations for 60 days. Exposure concentrations of total Se were 0.00, 0.171, 0.341, 0.683, 1.38, and 2.73 mg/L. All other exposure procedures were identical to those used in Exposure A. Mortality was monitored daily and samples for growth (length and weight) were taken at 0, 15, 30, and 60 days. Behavioral reponses were determined in the same manner as described for Exposure A above. Samples for Se tissue residue analyses were taken at 30 and 60 days, and at 60 days surviving fish were placed in Se-free water and sampled at 3, 7, 14, and 28 days to determine Se depuration rates.

In the third study (Exposure C) 3-month-old bluegill weighing about 0.2 g were fed a Se-laden diet for 90 days. The diet was a "Oregon moist", salmon-meal based, 1/8 inch pellet feed that was prepared by Department of Food Science and Technology, Oregon State University, Astoria, OR. A bulk quantity of the base diet was prepared. The exposure diets were then prepared by adding aqueous dilutions of seleno-L-methionine (Sigma Chemical Company, St. Louis, Missouri) to portions of the base diet to yield nominal total Se concentrations of 26.0, 13.0, 6.5, 3.25, and 1.63 mg/kg wet weight. Analysis of the diets showed that they actually contained 25.0, 12.7, 6.6, 3.5, and 2.3 mg Se/kg on a wet weight basis. The base diet with no added seleno-L-methionine contained 0.68 mg Se/Kg (wet weight), and was used as a control diet. Prior to starting the study, the bluegill were acclimated to the control diet over a one-week period, and then to exposure water and test temperature (25°C) over a 4-day period. Based on 0-day growth samples, the bluegill were fed the test diet at a rate of 3% of the biomass per test chamber per day. Feeding rate was adjusted to the control treatment biomass after each growth sampling period. Exposure water from each treatment was analyzed to determine if it contained Se that leached from the diet. Sampling procedures in this study were the same as those described above for Exposures A and B except that behavioral responses were determined only after 30 days of exposure.

3) Bluegill reproduction study: Two-year-old pond-cultured bluegill were treated prophylactically with 6 g/L NaCl for one week. During treatment and acclimation, fish were held in well water (pH, 7.8; hardness, 283 mg/L as $CaCO_3$; alkalinity, 255 mg/L as $CaCO_3$) at 22°C. The study was initiated by distributing 28 adult bluegill into each of 12 stainless steel (630 liter) exposure tanks of a modified proportional diluter. During the first 24 hours of the study, fish were exposed to 5 duplicated treatments containing 10 μg/L total Se (a 6:1 mixture of selenite and selenite) established in laboratory well water. Fish were also exposed to duplicated control conditions containing no waterborne Se. Total waterborne Se concentrations of the 6:1 mixture were 8.6 and 1.4 μg/L of selenate and selenite, respectively. After the initial 24-hour period, the generic fresh-water (described above) was substituted for the well water, and used for the duration of the study.

Bluegill in two treatments were fed control diet (containing no seleno-L-methionine). Fish in one of the two treatments receiving control diet were also exposed to 10 μg/L waterborne Se. Fish in the four remaining treatments were exposed to 10 μg/L of waterborne Se and fed one of four seleno-L-methionine diets. Mean measured seleno-L-methionine concentrations in the diets were 33.3. 16.8, 8.45 4.63 μg/g (dry weight). The adult bluegill were exposed to this treatment regime for a total of 140 days.

The first 60 days of the 140-day adult exposure comprised the pre-spawning phase of the study. During this period, lighting and temperature was controlled so as not to elicit spawning in the adult fish. A 12:12-hour light to dark photoperiod was maintained and the temperature was held at 22°C. Near the end of the pre-spawning phase, the photoperiod was adjusted to a 16:8-hour light to dark photoperiod, and the temperature was gradually increased to 28°C to achieve optimum conditions for bluegill spawning. During the second 90 days of the study (spawning phase), photoperiod and temperature was maintained to sustain the optimum spawning conditions for the fish.

During the spawning phase, the effects of water-borne and dietary Se on the reproductive success of bluegill was determined by measuring the frequency of spawning, number of eggs per spawn, hatching success of selected eggs, and survival of resultant fry for 30 days. The number of eggs per spawn was determined by removing the eggs from the substrate, measuring the total volume of eggs in the spawn and then counting a 10 percent volume of the eggs using a Biotran[R] colony totalizer. The number of eggs per volume of subsample was multiplied by the total volume of eggs in the spawn to estimate the total number of eggs per spawn.

The hatching success of eggs from each spawn was determined throughout the spawning phase of the study. After the eggs were carefully removed from the substrate, they were transferred to a glass dish, and examined with a dissecting scope for evidence of embryonic differentiation. Twenty viable eggs were then selected and transferred with an eye dropper into water in each of four hatching cups. The cups were then suspended in the adult exposure system in the treatment where the spawn occurred until the eggs hatched (approximately 16 hours post deposition). After hatching, the number of living and dead fry was recorded.

To determine fry survival for selected spawns, five fry were removed from each hatching cup (20 fry per spawn) and transferred to a growth chamber in a second diluter with a waterborne Se concentration corresponding to the parental exposure. Four sequential spawns from each treatment group of adult bluegills were used to evaluate fry survival during a 30-day period. The fry were automatically fed brine shrimp ad libitum every 30 minutes. At day 15 post-hatch, the brine shrimp was suplemented with the seleno-L-methionine-laden diet corresponding to the fry's parental diet treatment. Fry mortality was recorded daily. The length of fry was determined photographically on day 15 post-hatch, and at day 30 post-hatch, surviving fish were weighed, measured for total length, and frozen for whole-body Se residue analyses.

Water and tissue analyses for selenium: All samples were analyzed by hydride generation chemistry combined with atomic absorption spectrophotometry (hydride-AA). Selenite concentrations were determined by analyzing water samples directly, whereas samples requiring total Se measurements were subjected to a two-step persulfate digestion procedure (Presser and Barnes, 1984) to oxidize all Se species to the +6 oxidation state. Hot hydrochloric acid reduction was then used to reduce all Se to the +4 oxidation state required for hydride-AA. Selenate concentrations were calculated as the difference between "total" Se concentrations and selenite concentrations (total Se - selenite = selenate). Water samples spiked with selenomethionine were analyzed in the same manner as described above for total Se analyses. Samples of lyophilized fish tissue and selenomethionine-laden feed were subjected to a nitric/hydrochloric acid wet digestion and magnesium nitrate dry-ash procedure (Siu and Berman, 1984; Pettersson et al., 1986; Hansson et al., 1987), and then reduced with hot hydrochloric acid for analysis with hydride-AA as described above.

Quality assurance: The integrity of the data obtained from exposure water analyses was monitored by use of quality control protocols. Recoveries of Se from National Bureau of Standards (NBS) 1643b water averaged 100 percent (range = 95 to 102 percent; n=12). The percent relative standard deviation (RSD) for samples that were prepared and analyzed in triplicate provided an estimate of method precision. Percent RSD for triplicate sample analyses averaged 1.6 (range = 0.39 to 5.9; n = 11 triplicate groups). Recoveries of Se from digested spike solutions averaged 97.1 percent (range = 89 to 105 percent; n = 43). Recoveries of Se from spiked samples that were analyzed to check for matrix suppression or enhancement averaged 97.5 percent (range = 93 to 102 percent, n =13). Limits of detection (LOD) for Se in exposure water samples ranged from 0.008 to 0.035 μg/mL for total Se; from 0.030 to 0.038 μg/mL for selenate; and was 0.000018 μg/mL for selenite. Blank equivalent concentrations of Se in all precedural blank samples were less than the calculated limits of detection.

Quality control samples were also analyzed to confirm the integrity of analytical results obtained for samples of fish tissue and Se-laden diet. The concentrations of Se in NBS 1577 bovine liver were 1.05 ± 0.03 μg/g (mean ± standard deviation; n = 9) which were within the certified range of 1.0 to 1.2 μg/g. Se concentrations in NBS RM 50 tuna were 3.57 ± 0.12 μg/g (mean ± standard deviation; n = 9) which were also within the recommended range of 3.2 to 4 μg/g. Mean percent RSD, computed as described above for water samples, was 2.01 (range = 0.86 to 4.79 percent). Recovery of Se from digested spikes for tissue and blank samples averaged 98 percent (range = 84 to 120 percent). The

LOD for Se in tissue and diet samples averaged 0.076 μg/g (range = 0.014 to 0.283 μg/g). Blank equivalent concentrations of Se in all procedural blank samples were at or below the corresponding limit of detection. Recovery of Se from analytical spikes averaged 100 percent (range = 92 to 100 percent).

Statistical analyses: ANOVA was used to test for significant differences in percent mortality (arcsine transformed values), growth, and swimming (arcsin tranformed values) behaviors among the various treatments. Statistical analyses were performed with Statistical Analysis System (SAS, 1982) computer programs. Contrasts among means were performed by Fisher's Least Significant Difference (LSD) or Duncans Multiple Range tests.

Principal Findings

1) Acute waterborne Se toxicity studies: Measured exposure concentrations of Se at the initiation of each acute toxicity study were approximately 95% of nominal values. Selenium analyses at the end of the studies indicate that the exposure concentrations did not decline substantially during the 96-hour exposure period except for the selenomethionine studies in which nominal values decreased by 65-75 percent. The acute toxicity of Se in freshwater and ASTM soft water was similar. Although the exposures of bluegill to Se in the two different water qualities were not designed to determine the effect of sulfate, hardness, and alkalinity on toxicity, these parameters did not appear to influence the toxicity of Se.

The 96-hour LC50's (Table 1) show that selenate is less toxic to bluegill than selenite which is less toxic than both seleno-L-methionine and seleno-DL-methionine. The increased toxicity of the organic forms of Se compared to the inorganic forms may be related to differences in bioavailability or potential in vitro or in vivo transformation products. The acute toxicity to bluegill of the 6:1 mixture of selenate to selenite was intermediate between that of the inorganic forms singly and the organic forms of Se. These results imply that additive interactions of the two inorganic forms may occur in bluegill.

Table 1. Acute toxicity as 96-hour LC50's and 95% confidence interval (mg/L Se) of various forms of selenium to bluegill. Studies were conducted in both freshwater and ASTM soft water.

	Type of Exposure Water	
Selenium Form	Freshwater	ASTM
Selenate	72-120a	98 (80-119)
Selenite	7.8-13.0[a]	7.8-13.0[a]
Seleno-L-methionine	0.009 (0.007-0.011)	0.013 (0.011-0.016)
Seleno-DL-methionine	0.010 (0.008-0.012)	0.013 (0.011-0.016)

[a] Estimated range within which 96-hour LC50 occurs.

2) Chronic waterborne and dietary Se toxicity studies:

Water quality - The measured water quality parameters during the waterborne Se exposures (exposures A and B) and dietary exposure (exposure C) were close to the nominal values. During exposures A and B, mean water quality values for the Se treatments ranged from 7.9 to 8.3 for pH; from 69 to 72 mg/L as $CaCO_3$ for alkalinity, from 137 to 143 mg/L as $CaCO_3$ for hardness; from 66 to 94 mg/L for sulfate, and from 6 to 10 mg/L for chlorides. During exposure C, mean water quality values for the Se treatments ranged from 7.9 to 8.0 for pH; from 68 to 70 mg/L as $CaCO_3$ for alkalinity;

from 137 to 140 mg/L as CaCO3 for hardness; from 69 to 72 mg/L for sulfate; and from 5.6 to 6.0 mg/L for chloride. The measured waterborne Se concentrations during exposures A and B were close to the expected values (Table 2).

Table 2. Mean measured Se concentrations ± standard deviations (in parentheses) during aqueous exposures A and B.

Nominal Se (mg/L)	Measured Se mg\L		
	Total Se	Selenate	Selenite
Exposure A			
0.00	0.0095 (0.002)	---	---
1.4	1.2 (0.05)	---	---
2.7	2.5 (0.07)	---	---
5.4	4.9 (0.01)	---	---
10.9	10.1 (0.40)	---	---
21.7	21.2 (0.40)	---	---
Exposure B			
0.00	0.02 (0.01)	—	—
0.17	0.16 (0.01)	0.13 (0.005)	0.018 (0.002)
0.34	0.33 (0.01)	—	—
0.68	0.64 (0.01)	0.55 (0.00)	0.089 (0.01)
1.4	1.12 (0.44)	—	—
2.7	2.8 (0.10)	2.31 (0.11)	0.38 (0.035)

Growth and mortality - Bluegill exposed to 1.4 mg/L of water-borne Se in Exposure A incurred significant mortality (Table 3). Total mortality of bluegill exposed to 5.4 mg/L Se occured between 15 and 29 days, and fish exposed to concentrations of Se greater than or equal to 10.9 mg/L incurred total mortality between 0 and 15 days. No significant effects on growth were observed in bluegill that survived for 29 days at Se concentrations less than or equal to 2.7 mg/L (Table 3). At 60 days of Exposure B, a chronic no-effect concentration between 0.34 and 0.68 mg/L of waterborne Se was determined from bluegill mortality (Table 4). During Exposure B, significant effects on the growth of bluegill were limited to a reduction of condition (K) after 30 days of exposure to 2.7 mg/L Se (Table 4). Although not statistically significant, A trend toward increased growth was observed in all the Se treatments after 60 days during Exposure B (Table 4). During Exposure C, significant dose-related effects on the mortality or growth of bluegill were not observed at any of the dietary Se concentrations tested. At 60 days of Exposure C, significant mortality ocurred in bluegill exposed to 6.5 and 26 mg Se/Kg, but not in those exposed to 13 mg Se/Kg (Table 5). At 90 days only bluegill exposed to 6.5 mg Se/Kg incurred significant mortality compared to the control treatment. At 90 days significant decreases in the condition of bluegill occured at dietary Se treatments of 13 and 26 mg/Kg (Table 5).

Table 3. Mean and standard deviation (in parentheses) of mortality, length, weight, and condition factor (K) for juvenile bluegill during exposure to aqueous Se for 29 days. Asterisks denotes significant difference from controls within time periods ($P \leq 0.05$, LSD Test).

Days of exposure and parameter	Nominal exposure concentration (mg/L)					
	Control	1.4	2.7	5.3	10.9	21.7
15 days						
Mortality (%)	5.0	15.0*	22.5*	75.0*	100*	100*
	(10.0)	(19.1)	(15.0)	(20.8)	(0.00)	(0.00)
Length (mm)	27.6	27.7	26.6	26.7	--	--
	(3.2)	(2.7)	(2.0)	(2.3)	--	--
Weight (g)	0.28	0.27	0.24	0.26	--	--
	(0.14)	(0.11)	(0.08)	(0.09)	--	--
Condition (K)	1.2	1.2	1.2	1.3	--	--
	(0.17)	(0.16)	(0.19)	(0.15)	--	--
29 days						
Mortality (%)	7.5	50.0*	87.5*	97.5*	--	--
	(9.6)	(23.4)	(12.6)	(5.0)	--	--
Length (mm)	29.8	30.3	28.7	--	--	--
	(4.7)	(4.1)	(1.5)	--	--	--
Weight (g)	0.39	0.41	0.32	--	--	--
	(0.26)	(0.21)	(0.06)	--	--	--
Condition (K)	1.3	1.4	1.4	--	--	--
	(0.17)	(0.22)	(0.36)	--	--	--

Table 4. Mean and standard deviation (in parentheses) of mortality, length, weight, and condition factor (K) for juvenile bluegill exposed to aqueous Se for 60 days. Asterisks denotes significant difference from controls within time periods (P≤ 0.05, LSD Test).

Days of exposure and parameter	Nominal exposure concentration (mg/L)					
	0.00	0.17	0.34	0.68	1.4	2.7
15 days						
Mortality (%)	0.0	0.0	7.5	15.0*	25.0*	47.5*
	--	--	(9.6)	(12.9)	(25.2)	(17.1)
Length (mm)	33.2	32.2	33.3	32.1	32.4	32.8
	(3.1)	(2.7)	(3.7)	(3.5)	(3.3)	(2.8)
Weight (g)	0.49	0.44	0.52	0.43	0.44	0.47
	(0.15)	(0.12)	(0.18)	(0.16)	(0.15)	(0.14)
Condition (K)	1.3	1.3	1.3	1.3	1.3	1.3
	(0.11)	(0.10)	(0.14)	(0.14)	(0.26)	(0.13)
30 days						
Mortality	0.0	10.0	20.0*	40.0*	55.0*	87.5*
	--	(14.1)	(18.3)	(27.1)	(23.8)	(12.6)
Length (mm)	35.7	35.4	37.8	35.8	35.2	36.7
	(4.2)	(4.1)	(4.0)	(5.7)	(5.1)	(5.9)
Weight (g)	0.68	0.65	0.82	0.72	0.64	0.67
	(0.25)	(0.25)	(0.29)	(0.35)	(0.33)	(0.43)
Condition (K)	1.4	1.4	1.5	1.4	1.3	1.2*
	(0.13)	(0.23)	(0.12)	(0.17)	(0.20)	(0.23)
60 days						
Mortality (%)	10.0	12.5	22.5	52.5*	70.0*	97.5*
	(20.0)	(18.9)	(15.0)	(35.9)	(28.3)	(5.0)
Length (mm)	40.4	40.4	42.7	41.9	43.9	--
	(5.7)	(5.7)	(5.5)	(6.3)	(5.5)	--
Weight (g)	1.0	1.1	1.3	1.2	1.4	--
	(0.4)	(0.5)	(0.5)	(0.4)	(0.5)	--
Condition (K)	1.5	1.5	1.6	1.5	1.6	--
	(0.1)	(0.2)	(0.3)	(0.1)	(0.1)	--

Table 5. Mean and standard deviation (in parentheses) of mortality, length, weight, and condition factor (K) for juvenile bluegill exposed to Se laden feed for 90 days. Asterisks denotes significant difference from controls within time periods ($P \leq 0.05$, LSD Test).

Days of exposure and variable	Nominal seleno-L-methionine in feed (mg/Kg)					
	Control	1.6	3.3	6.5	13.0	26.0
30 days						
Mortality (%)	2.5	5.0	5.0	10.0	12.5	5.0
	(5.0)	(10.0)	(5.8)	(8.2)	(12.6)	(5.8)
Length (mm)	30.4	30.7	30.5	30.4	29.4	29.4
	(3.7)	(4.2)	(3.4)	(4.0)	(3.7)	(4.1)
Weight (g)	0.40	0.45	0.42	0.44	0.39	0.36
	(0.18)	(0.22)	(0.17)	(0.18)	(0.18)	(0.18)
Condition (K)	1.5	1.4	1.4	1.4	1.4	1.3*
	(0.1)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)
60 days						
Mortality (%)	2.5	5.0	7.5	22.5*	12.5	17.5*
	(5.0)	(10.0)	(9.6)	(5.0)	(12.6)	(9.8)
Length (mm)	34.3	33.6	34.9	33.9	33.2	34.3
	(5.4)	(5.8)	(5.5)	(5.8)	(5.9)	(6.6)
Weight (g)	0.63	0.60	0.69	0.62	0.57	0.65
	(0.31)	(0.38)	(0.35)	(0.35)	(0.33)	(0.44)
Condition (K)	1.5	1.4	1.5	1.4	1.4	1.4
	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
90 days						
Mortality (%)	5.0	7.5	10.0	22.5*	15.0	17.5
	(5.8)	(9.6)	(14.1)	(5.0)	(10.0)	(9.6)
Length (mm)	40.4	38.7	40.8	38.5	38.5	39.1
	(6.9)	(8.1)	(6.4)	(8.0)	(8.1)	(9.8)
Weight (g)	0.95	0.87	0.97	0.84	0.81	0.91
	(0.53)	(0.62)	(0.51)	(0.59)	(0.54)	(0.8)
Condition (K)	1.3	1.3	1.3	1.3	1.2*	1.2*
	(0.09)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)

Behavior - Aqueous selenium exposure induced behavioral changes in bluegills. During exposure A a number of abnormal responses were evident after the first week of exposure including the inhibition of feeding and abnormal swimming among fish exposed to Se concentrations ranging from 5.4 to 21.7 mg/L (Figure 1). At day 10 of exposure, aggressive responses were inhibited among these treatments, and feeding behavior was inhibited among fish exposed to 1.4 mg/L Se (Figure 1). Quantitative measures of swimming behavior after three weeks of exposure revealed hypoactivity among fish exposed to 2.7 mg/L Se (Table 6).

Table 6. Swimming behavior of bluegill exposed to different concentrations of aqueous selenium for 21 days during exposure A. Means within a column accompanied by different letters are significantly different.

Nominal selenium concentration (mg/L)	Swimming Activity Variables	
	duration[1]	frequency
Control	18.9 (3.3)	12.2 (1.0)a
1.4	20.4 (3.3)	14.4 (1.5)a
2.7	10.6 (3.3)	7.8 (1.0)b

[1] Behaviors are reported as mean and standard error in parenthesis for duration of activity (sec), and frequency of activity (cumulative moves).

Within the first week of exposure B the behavioral surveys detected abnormal swimming behavior that included lethargic, abnormal swimming postures, and confinement to the aquaria bottoms among bluegill exposed to Se concentrations ranging from 0.68 to 2.7 mg/L (Figure 2). Further quantification of swimming behavior after 18 days of exposure (Table 7) revealed significant reductions in the frequency and duration of swimming movements relative to controls among all Se-treated fish except those exposed to 0.68 mg/L. Trends of reduced speed and distance of movement were also found among fish exposed to 2.7 mg/L Se (Table 7).

Table 7. Swimming behavior of bluegill following exposure to different concentrations of aqueous selenium for 18 days during exposure B. Means within a column accompanied by different letters are significantly different.

Nominal selenium concentration (mg/L)	Swimming Behavior Variables			
	duration[1]	speed	distance	activity
Control	9.7 a (1.0)	2.8 a[2] (0.8)	186 a[2] (54)	11.8 a (0.5)
0.17	12.5 bc (1.0)	3.5 ab (0.8)	229 ab (58)	9.8 b (0.5)
0.34	13.9 bc (1.0)	2.7 a (0.8)	296 ab (54)	9.9 b (0.5)
0.68	17.9 a (1.0)	5.9 b (0.8)	390 b (56)	12.5 a (0.5)
1.4	16.0 ab (1.0)	4.1 ab (0.8)	270 ab (57)	9.3 b (0.5)
2.7	12.7 b (1.4)	1.2 a (1.2)	82 a (83)	5.3 c (0.7)

[1] Behaviors are reported as mean and standard error in parenthesis for duration of activity (sec), average speed per video frame, distance traveled per frame x 100, and frequency of activity (cumulative moves).

[2] Means for speed and distance significant by unprotected ($P \geq 0.05$) LSD tests.

The behavioral survey and measurement of swimming activity variables at day 30 of exposure did not detect any significant behavioral changes induced by dietary Se. Fish of each treatment responded similarly to controls throughout the dietary exposure (Table 8).

Table 8. Swimming behaviors of bluegill exposed to dietary selenium for 30 days during exposure C.

Nominal dietary seleno-L-Methionine (mg/kg)	Swimming Behavior Variables			
	duration[1]	speed	distance	activity
Control	14.2 (2.8)	5.3 (0.7)	348 (44)	14.9 (2.2)
1.6	13.9 (3.0)	3.4 (0.6)	223 (41)	16.3 (2.3)
3.3	14.8 (3.0)	4.5 (0.7)	298 (43)	14.0 (2.3)
6.5	16.9 (2.5)	3.3 (0.6)	219 (42)	14.9 (1.9)
13.0	15.6 (2.5)	4.0 (0.6)	263 (39)	13.5 (1.9)
26.0	12.7 (2.2)	3.5 (0.6)	229 (40)	16.1 (1.7)

[1] Behaviors are reported as mean and standard error in parenthesis for duration of activity (sec), speed per frame, distance traveled per frame x 100, and frequency of swimming activity (cumulative moves).

Bioconcentration - The uptake of Se from both water (Figure 3) and diet (Figure 4) was directly related to exposure concentration. Bioconcentration factors ranged from 5 to 17 in water and from 0.5 to 1.0 in diet. During depuration, bluegill eliminated most of the accumulated Se regardless of exposure route (Figures 3 and 4). However, at 14 days of depuration, Se in the tissue of some of the exposed groups were still significantly higher than that of controls.

3) Reproduction study:

Residue analyses - Selenium accumulation in whole fish during the first 56 days, and at day 140 of the reproduction study is presented in Figure 5. The Se tissue concentrations were directly related to Se exposure concentrations. Figures 6 shows Se concentrations in carcass and gonadal tissue at day 60 (end of the pre-spawning period) and day 140 (end of the study). These data indicate that Se tissue concentrations reached equilibrium during the pre-spawning period of exposure. Ovaries accumulated more Se than either carcass or testes. Se concentrations in eggs collected from the spawning substrates were similar to ovarian Se concentrations indicating that Se was deposited in the the eggs during oogenesis rather than during aqueous exposure in the spawning substrates (Figure 7).

Biological effects - Exposures of 140 days to dietary selenomethionine up to 33.3 μg/g (dry weight) in combination with 10 μg/L total Se in the exposure water did not adversely effect the weight, length, gonadal weight, gonasomatic index (gonad weight/body weight), hematocrit, spawning frequency, or fecundity of adult bluegill. The percent hatch of eggs and the growth of surviving fry were also unaffected.

The influence of adult exposure to dietary Se on the survival of resulting fry was the most significant biological effect observed during the study. The mean survival to day 30 of fry from all spawns collected during the exposure is shown in Figure 8. Although fry survival within and between exposure groups varied considerably, fry resulting from parents that spawned in the highest combination of water-borne and dietary Se (10 μg/L waterborne Se and 33.3 mg/Kg dietary Se) consistently failed to survive. In contrast to the other exposure groups, these fish exhibited almost total mortality prior to the critical period in their development when they switch from internal (yolk sac) feeding to external food sources.

These results suggest that exposure to Se combinations in the water and diet that may not elicit overt toxicological responses in adult fish, but may result in reproductive failure by severely reducing the survival of their offspring. Such a striking reproductive effect may help explain the mechanism by which fisheries are lost in Se-contaminated waters.

This study has contributed to a better understanding of the effect of Se on fish reproduction. In addition to documenting an apparent reproductive effect, the results of tissue residue analyses performed on fish with a known exposure history may provide a tool by which feral bluegill populations can be evaluated for Se-related reproductive effects.

Research Needs

Elucidation of selenomethionine toxicity - Microcosm studies conducted at the National Fisheries Contaminant Research Center indicate that selenomethionine is rapidly metabolized in non-sterile water presumably by microbiological activity. The toxicity observed in the acute studies with selenomethionine may have been caused by a breakdown product (i.e., methyl selenide) instead of the parent compound. Metabolic conversion of selenomethionine may explain the similarity in toxicity noted in the L vs DL selenomethionine studies, the high toxicity of selenomethionine, and the rapid loss of selenomethionine from acute exposure containers. Speciation studies performed during aqueous exposure of fish to selenomethionine could serve to identify specific compounds responsible for the observed toxicity.

Potential ameliorative effect of sulfate on Se toxicity - Due to the similarity between Se and sulfur the possibility exists that the presence of sulfates in water may reduce the toxicity of inorganic Se, particulary selenate, to aquatic organisms by competitive uptake. Acute studies conducted with fish exposed to waterborne Se concentrations near the toxic threshold in conjunction with varying levels of sulfate could demonstrate whether interaction between the two elements occur.

Evaluation of the toxicity of Selenocysteine - Most of the research that has been conducted to date on organoselenium focused on selenomethionine, but the possibility exists that other forms of organoselenium compounds exhibit appreciable toxicity. In particular, the toxicity of the selenoamino acid selenocysteine has not been investigated. Future research might assess the potential toxicity of this Se-containing compound and compare its toxicity to that of selenomethionine.

Improvement of methods for ensuring higher survival of bluegill fry - Methods for maximizing post-swim-up fry survival could be improved. Ensuring more uniform survival of fry from exposed parents, would provide a more statistically robust biological endpoint. Improvement of survival is undoubtedly related to providing adequately sized zooplankton to bluegill fry at the critical stage (about 7 days post hatch) when they switch from endogenous to exogenous feeding. Such a feeding regime could be developed by selecting, culturing and feeding various types of zooplankton to bluegill fry during the critical stage.

Expansion of dietary Se exposure range between 15 and 33 mg/Kg Se -

The results of the bluegill reproductive study suggests a threshold concentration exists between 15 and 33 mg/Kg (dry weight) of dietary Se, beyond which fry survival is severely diminished. Expansion of the Se exposure concentration range between 15 and 33 mg/Kg would provide a more precise estimate of a dietary no-effect concentration. Exposing bluegill to a series of dietary Se concentrations within this range would better define the dose-response curve for this contaminant.

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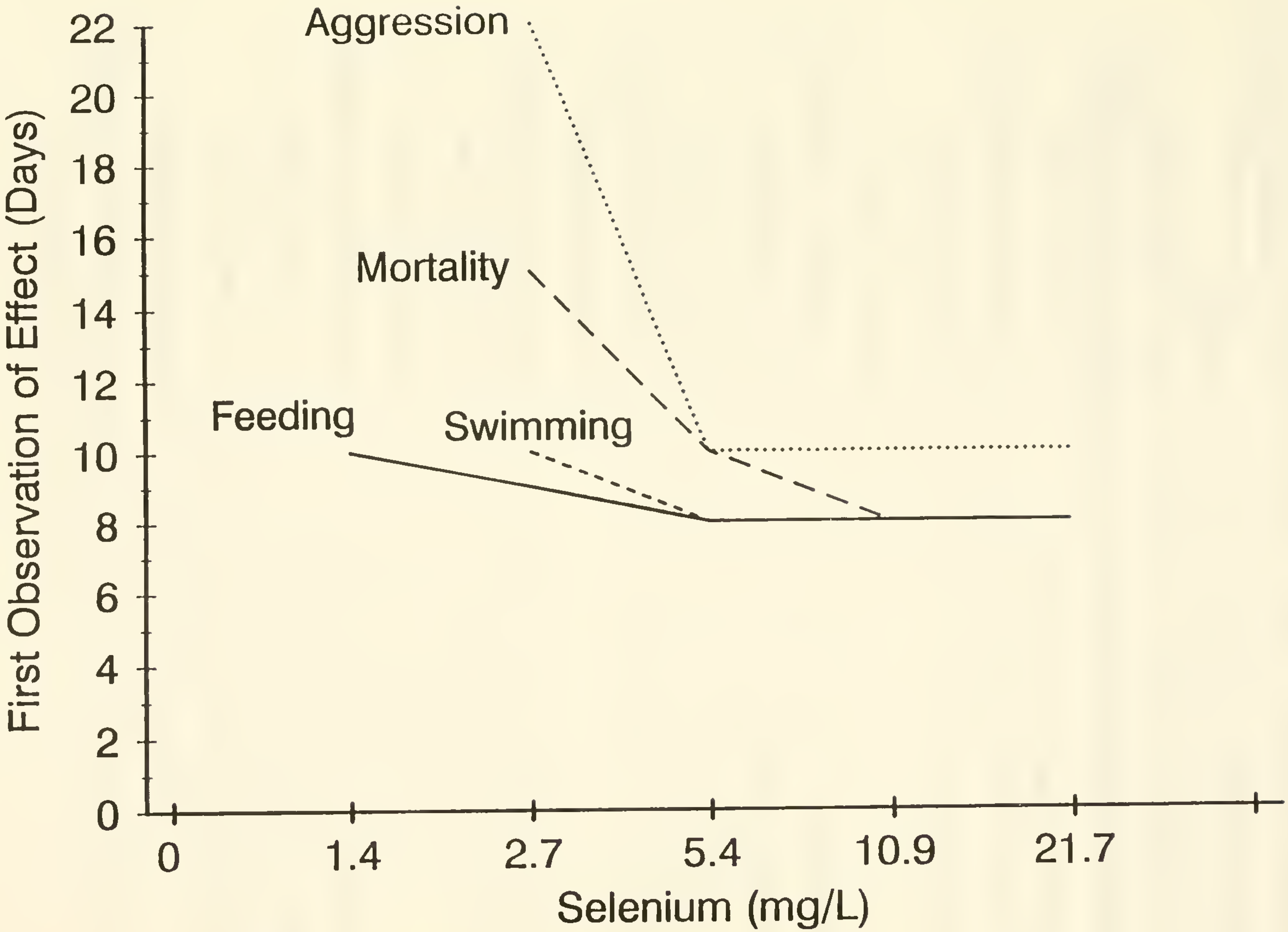

Figure 1. Swimming behavior of bluegill exposed to selenium during aqueous exposure A.

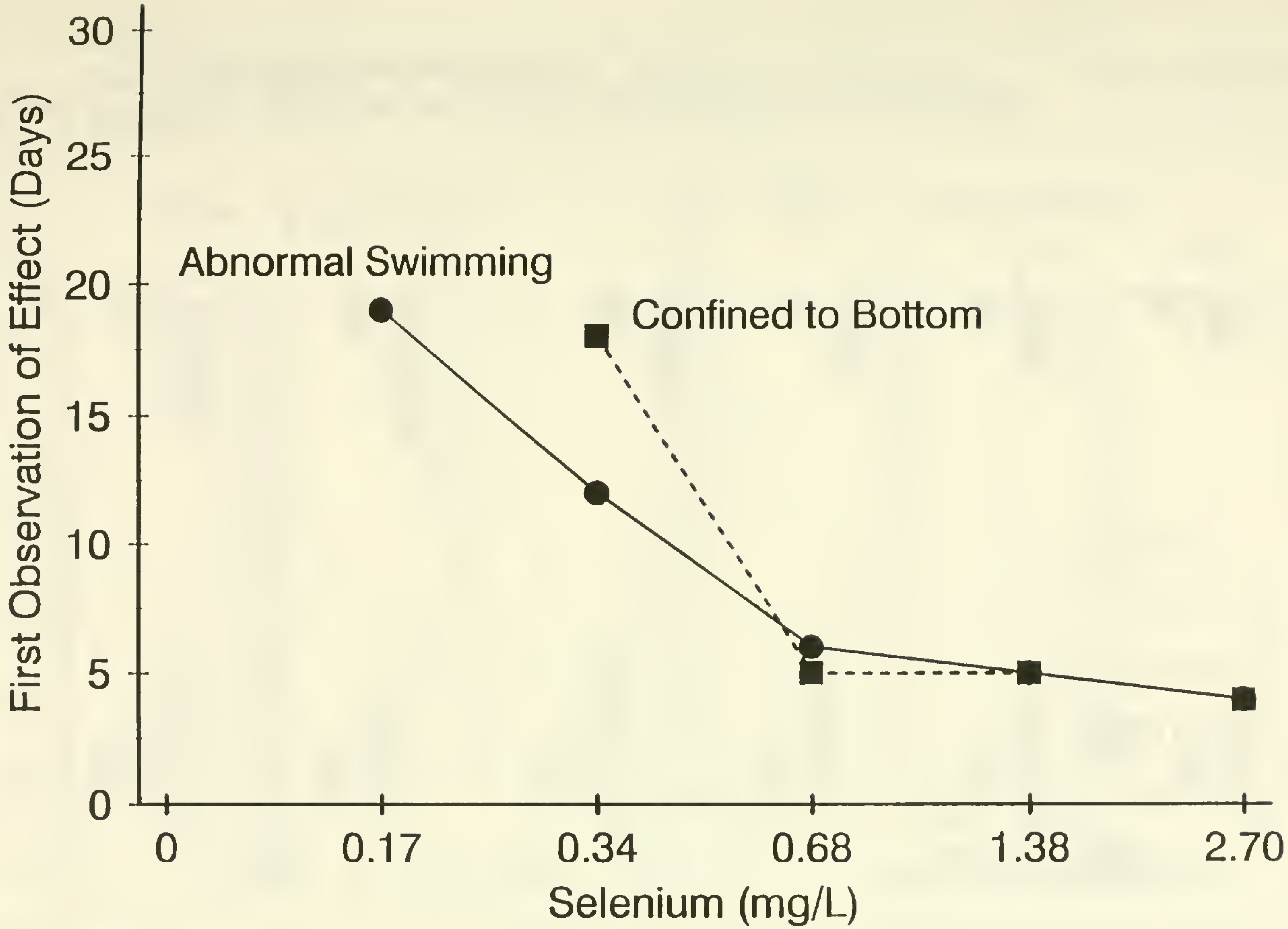

Figure 2. Swimming behavior of bluegill exposed to selenium during aqueous exposure B.

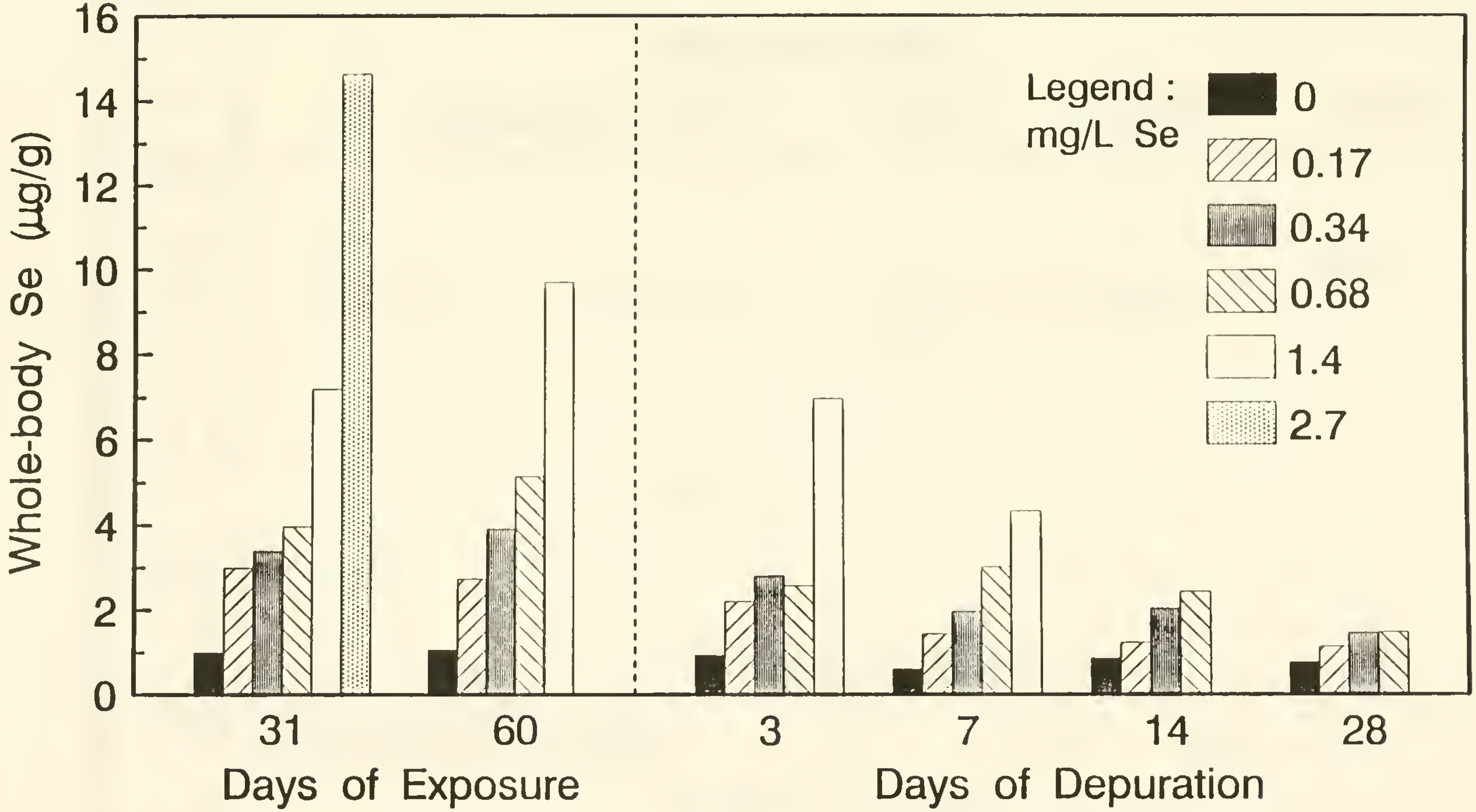

Figure 3. Bioconcentration and depuration of selenium by bluegill during aqueous exposure B.

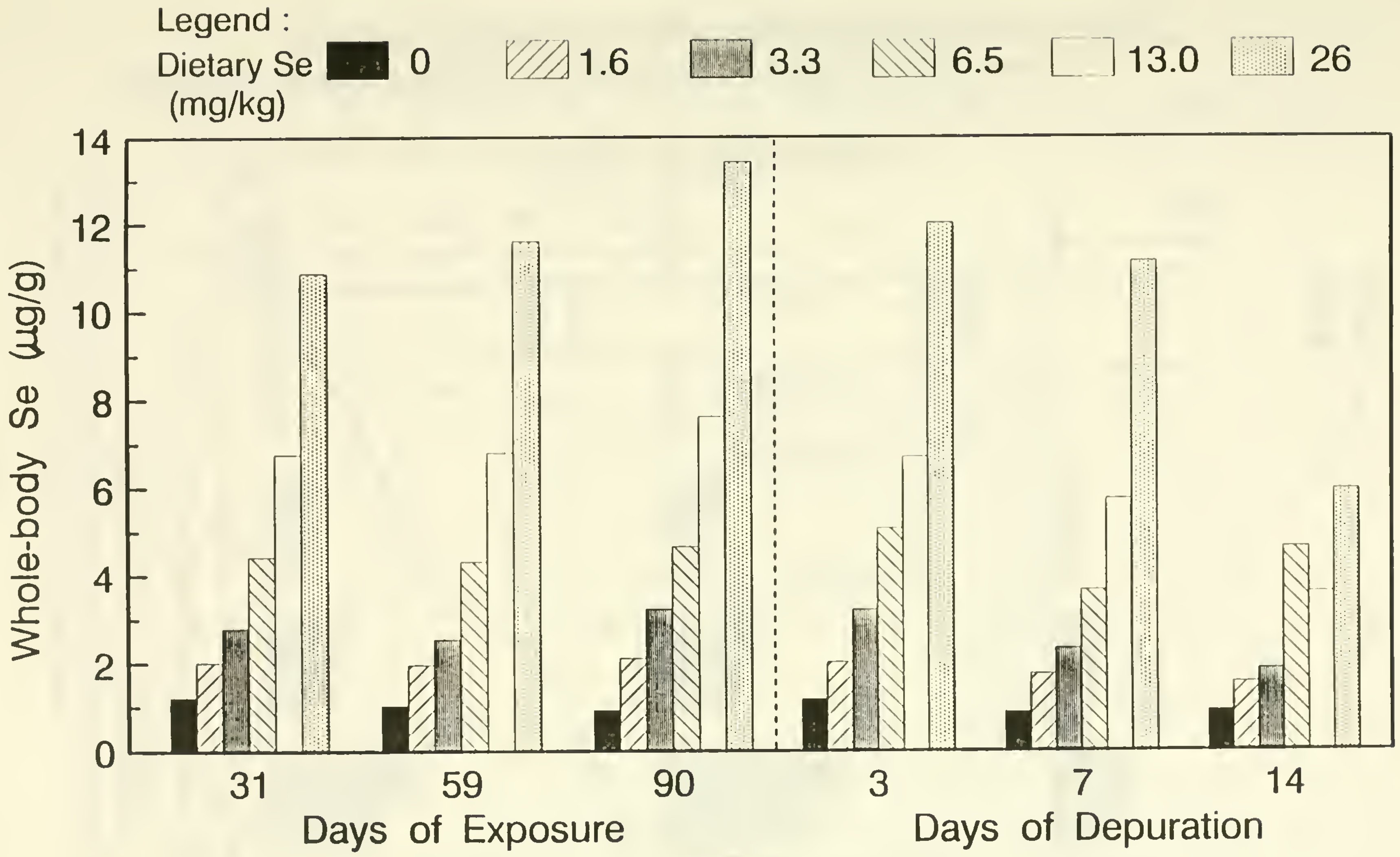

Figure 4. Bioconcentration and depuration of selenium by bluegill during dietary exposure C.

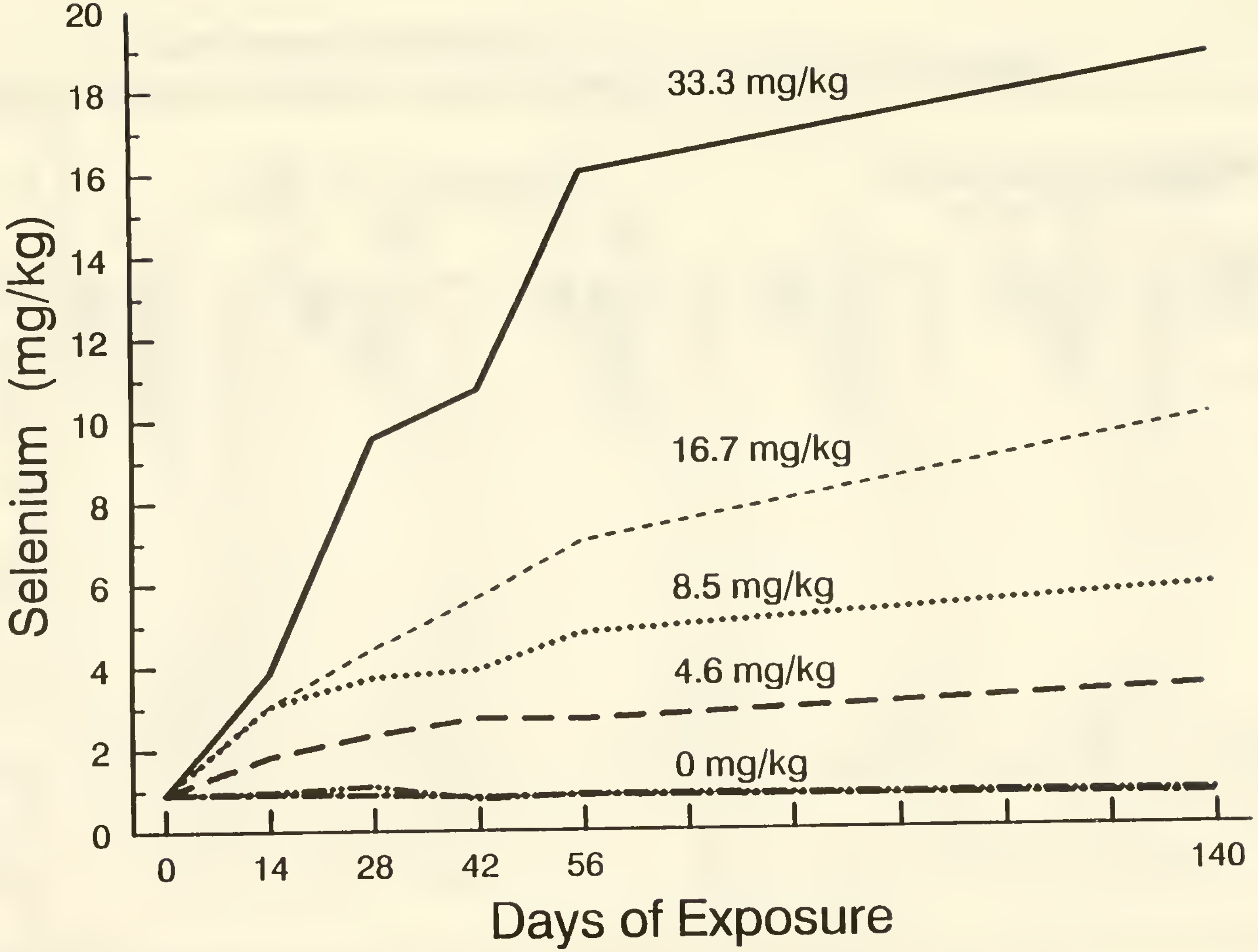

Figure 5. Bioaccumulation of selenium by adult bluegill during the first 56 days and at day 140 of the reproduction study.

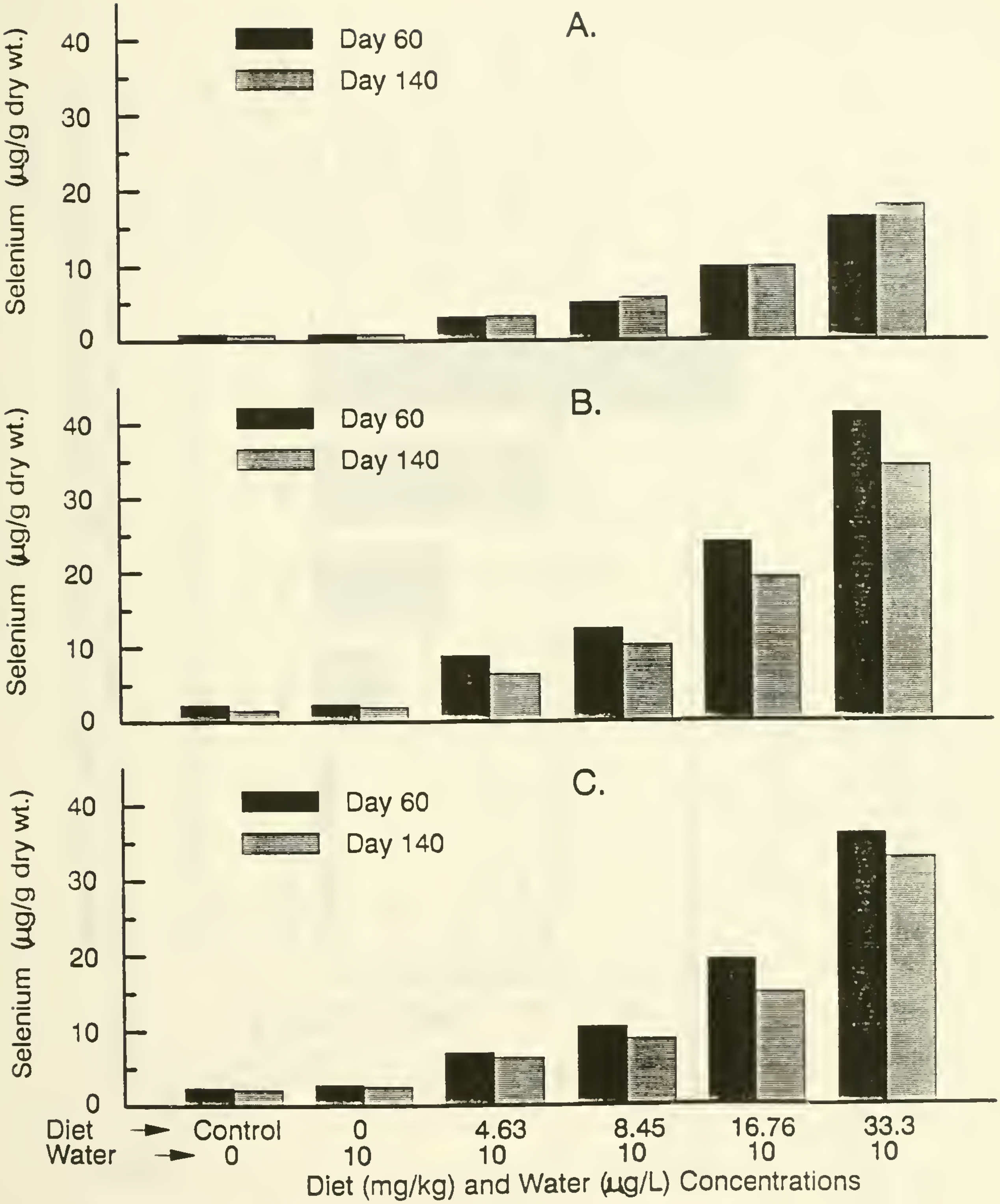

Figure 6. Bioaccumulation of selenium in A) carcasses, B) ovaries, and C) testes of adult bluegill after the 60 day prespawning period and the end of the 140 day reproduction study.

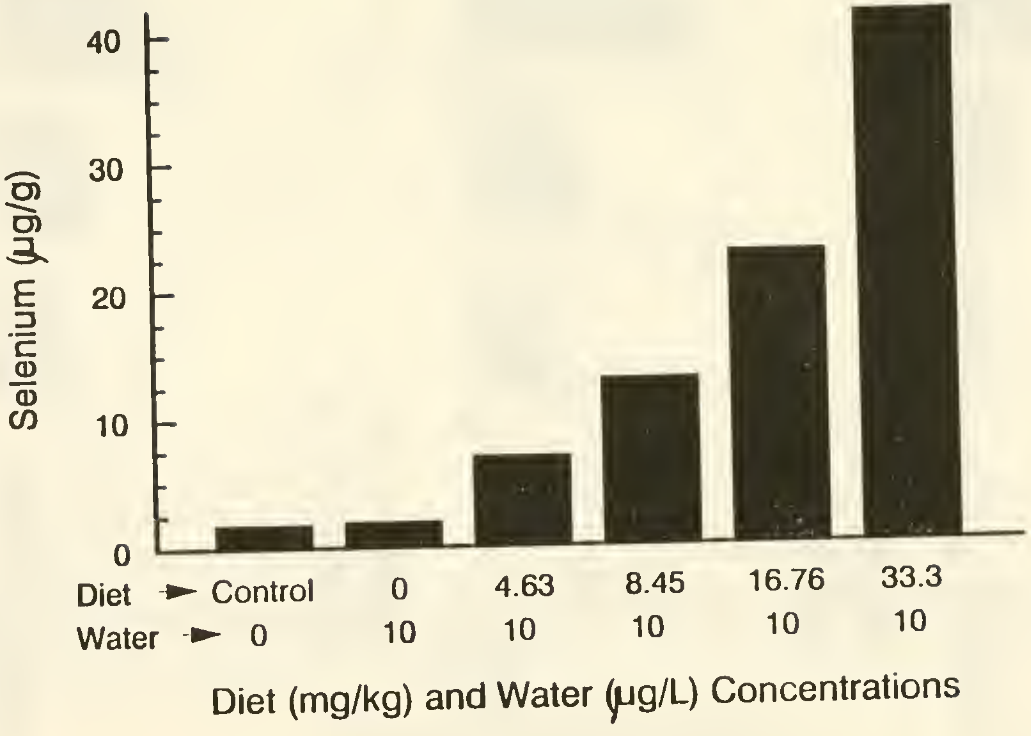

Figure 7. Bioaccumulation of selenium in eggs collected from the spawning substrates during the bluegill reproduction study.

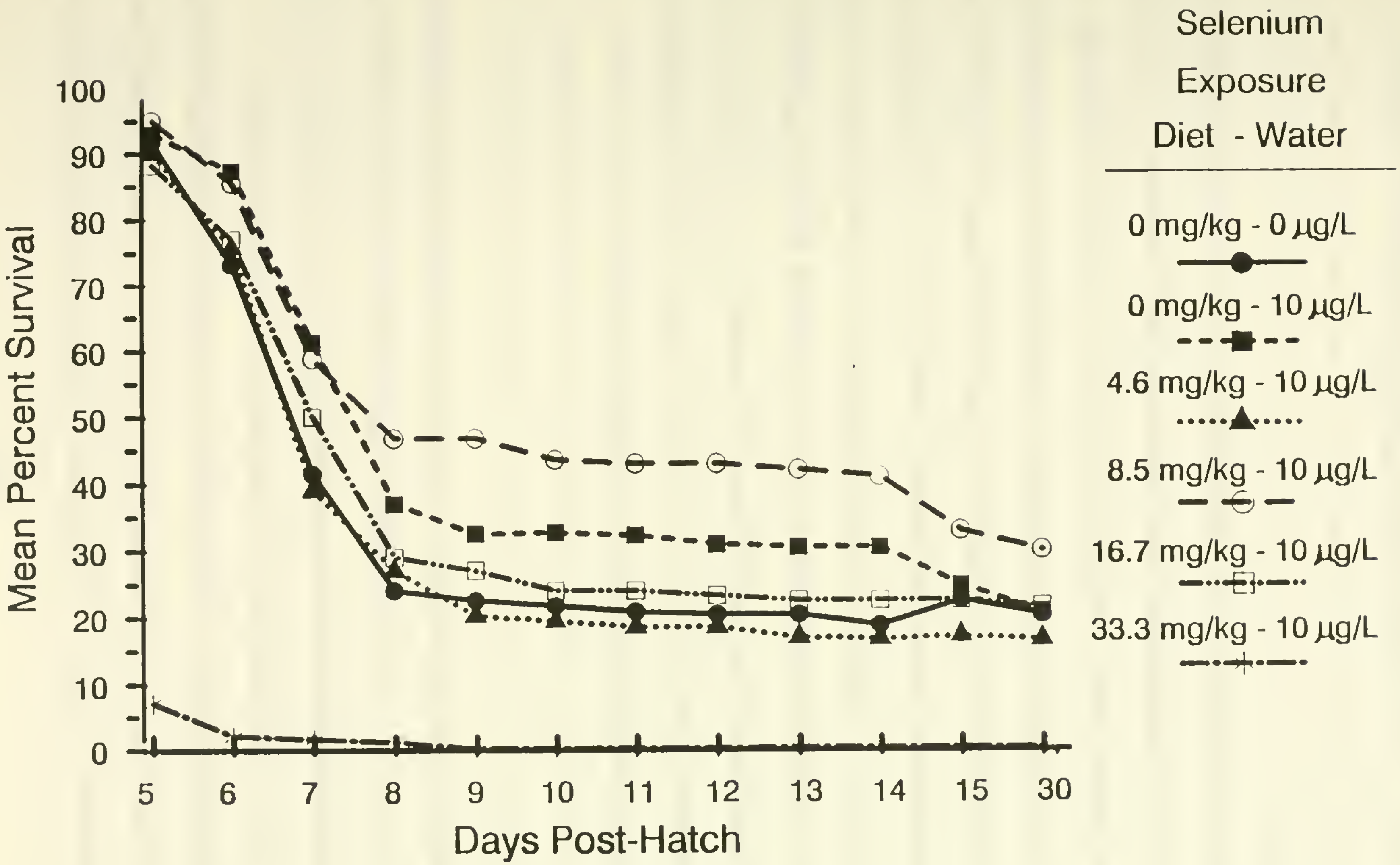

Figure 8. Mean survival to day 30 of bluegill fry from eggs spawned during the reproduction study.

LABORATORY AND MICROCOSM STUDIES OF SELENIUM FATE AND BIOACCUMULATION

Introduction

Field and laboratory research has found evidence of Selenium (Se) toxicity to fish at dissolved Se concentrations well below those having direct toxic effects (Cumbie and van Horn, 1979; Lemly, 1985). Selenium toxicity has increasingly been linked to Se concentrations in fish or in fish-food organisms, which accumulate Se via direct uptake of dissolved Se and transfer of Se residues in aquatic food chains. Inorganic Se species (selenite and selenate) are the predominant dissolved Se forms in contaminated aquatic environments, but Se residues in food-chain organisms may consist largely of organic Se compounds. Organoselenium species may be accumulated by aquatic organisms from low concentrations in water or produced in tissues by biotransformation of selenate or selenite.

The objective of these studies was to relate Se residues in fish to Se concentrations and speciation in water and Se dynamics in aquatic food chains.

Methods

We conducted two types of studies: microcosm Se fate studies, which evaluated factors affecting the formation and persistence of Se species in aquatic ecosystems; and food-chain Se-bioaccumulation studies, which compared the bioconcentration of Se species from water and the transfer of that accumulated to consumers in a simulated food chain.

Microcosm fate studies: The first fate study compared the influences of sediment characteristics and dissolved sulfate concentrations on the distribution and bioaccumulation of a simulated natural mixture of Se compounds. Each of 12 microcosms received one of two sediment types and one of two sulfate concentrations (3 microcosms for each combination of sediment type and sulfate concentration). Sources of the two sediment types were a mid-channel site in San Joaquin River near Los Banos, California ("San Joaquin" sediment), and a pond on the Volta Wildlife Area, California ("Volta" sediment). Volta sediment had a higher proportion of silt- and clay-sized particles, higher organic content, and higher nutrient levels than San Joaquin sediment. Atomic absorption analysis found low but detectable Se residues in the Volta sediment, but dissolved Se concentrations in control microcosms (which received sediments but no added Se) indicated no measurable release of Se from either sediment. Reconstituted test water with ionic composition similar to that of water in the San Joaquin River (pH 8.04, sulfate 74 mg liter^{-1}, alkalinity and hardness 70 and 136 mg liter^{-1} as $CaCO_3$, respectively) was added to microcosms without added sulfate ("low sulfate" treatment) or with 100 mg liter^{-1} added sulfate ("high sulfate" treatment). Microcosms in the first study received a mixture of three selenium species based on their proportions in contaminated drainwaters in the San Joaquin Valley: sodium selenate (Se^{+6}), 34 μg Se liter^{-1}; sodium selenite (Se^{+4}), 5.7 μg Se liter^{-1}; and seleno-L-methionine (Se^{-2}), 1.9 μg Se liter^{-1}. An equal activity of each ^{75}Se-labeled species (2 μCi each; 6 μCi per microcosm) was added to each microcosm to allow detection of trends in the fate and bioconcentration of each species.

The second fate study was designed to compare the fate and bioaccumulation of the three Se species. Nine microcosms were divided into three groups, each of which received only one of the three Se species as ^{75}Se (3 μCi of ^{75}Se per microcosm). Each microcosm received all three nonlabeled species to produce the same total Se concentrations and proportions of Se species used in the first study. All microcosms in the second study received reconstituted water (without added sulfate) and sediment from a depositional site in the San Joaquin River.

Microcosms in both studies were inoculated with algae (Selenastrum capricornutum) and stocked with daphnids (Daphnia magna) before they were dosed with ^{75}Se. The activity of ^{75}Se in samples of dissolved, suspended, and volatile phases was measured periodically during the studies. After 28 days, microcosms were dismantled to allow sampling of sediments and biota (macrophytes, periphyton, and daphnids).

Statistical analyses were conducted with SAS statistical software (SAS Institute 1987). Comparisons of Se distribution and bioaccumulation among treatments were made by analysis of variance (ANOVA); data were rank-transformed to equalize variances among treatments (Conover and Iman, 1980). Two-way rank ANOVA was used to determine effects of sediment and sulfate treatments in the first study; differences among Se compounds in the second study were tested with one-way rank ANOVA (Kruskal-Wallis test) with multiple comparisons of rank means made with Fisher's LSD procedure. Changes in dissolved Se concentrations over time were modeled with linear or polynomial regression (Neter and Wasserman, 1974). Statements of statistical significance refer to a 5% Type I error rate ($p \leq 0.05$).

Food-chain bioaccumulation studies: Bioaccumulation studies evaluated the influence of Se speciation on aqueous and food-borne Se bioaccumulation in a simulated aquatic food chain. Algae (C. reinhardtii), daphnids (D. magna), and fish (bluegill, Lepomis macrochirus) were exposed to three Se species over a range of concentrations: sodium selenate (Se^{+6}), 10-1,000 µg Se/l; sodium selenite (Se^{+4}), 1-100 µg Se/l; and seleno-L-methionine (Se^{-2}), 0.1-10 µg/l. Bioassays were conducted in an environmental chamber at 24 °C; photoperiod was 18 hr daylight:6 hr darkness for daphnids and bluegill, continuous light for algae. In exposures with aqueous Se, solutions of ^{75}Se-labeled ("hot") compounds (obtained from Amersham, Inc., Arlington Heights, Illinois) and nonradioactive ("cold") solutions were added to reconstituted exposure water (pH 8.0, hardness 136 mg/l, alkalinity 70 mg/l, sulfate 70 mg/l) to produce exposure solutions with the desired specific activity at the start of each study. Replacement exposure solutions added during the course of the studies were prepared with the same ratios of radioactive to nonradioactive stock solutions. In food-chain exposures, Se was introduced into the laboratory food chain via exposures of batch algae cultures to the same Se species and concentrations used in aqueous exposures. Radioactivity in samples collected during the studies was measured with a Beckman 8000 gamma counter and adjusted for decay of the ^{75}Se radioisotope, and Se concentrations in organisms were calculated on a dry-weight basis.

Algae exposures were conducted in 500 ml Ehrlenmeyer flasks for 48 hr under static conditions. Exposures with each of the three Se species consisted of three Se concentrations and a control, with three replicate flasks per treatment. Samples of algae and exposure solution were withdrawn from each flask periodically during the exposures. Daphnids in aqueous and food-chain Se exposures were held in 600 ml beakers for 4 and 14 days, respectively, and fed control or Se-labeled algae concentrates. Aqueous selenate and selenite exposures were conducted under static conditions; aqueous Se-methionine exposures and food-chain exposures were conducted with a static/renewal test system, which provided each beaker with two replacement volumes per day. Experimental designs for daphnid exposures were similar to those in algae exposures, except that three exposure beakers per dose level were harvested on each sampling date. Bluegill aqueous and feeding exposures were conducted with a static-renewal exposure system similar to that used for daphnids. Bluegill fry (approx. 8 weeks old) were stocked in one-liter beakers and fed daphnids which had been fed control or Se-dosed algae cultures. Aqueous and food-chain exposures were conducted simultaneously with each of the three Se species (Studies 1 and 2). Selenium species were dosed at equal, nominal doses (10 ug Se/l) in Study 1, to allow direct comparison of Se uptake, and were dosed at different concentrations in Study 2 (Se-methionine, 1 μg/l; selenite, 10 μg/l; selenate, 100 μg/l) to compare Se uptake from a ratio of these species more representative of natural systems. In these studies, individual fish were collected from each treatment periodically during the exposure period, and replicate samples were collected at the end of each exposure (37 days in Study 1 and 18 days in Study 2). Selenite-based exposures were conducted to compare Se uptake and depuration among aqueous, food-chain, and combined (aqueous + food-chain) exposure routes (Studies 3 and 4). Treatments consisted of three exposure routes, each at two dose levels (10 and 100 μg/l), with three replicate exposure chambers per treatment. Both studies consisted of a 30-day exposure period (with single fish collected from each chamber at 7, 14, 21, and 30 d) followed by a depuration period (14 days in Study 3, with samples collected at 7 and 14 days; and 30 days in Study 4, with samples collected at 15 and 30 days.

Statistical comparisons among bioaccumulation studies were made with procedures from the SAS software package (SAS Institute Inc. 1987). Comparisons among mean Se concentrations were made by analysis of variance on log-transformed data. Statements of statistical significance refer to a 5% Type I error rate ($p \leq 0.05$). Bluegill Se bioaccumulation were modeled with the BIOFAC computer program (Blau and Agin 1978), which is based on a simple two-compartment kinetic model. The BIOFAC program estimated uptake and depuration rates from both uptake-only exposures (algae and daphnid exposures and bluegill Studies 1 and 2) and combined uptake and depuration studies (bluegill Studies 3 and 4). In feeding exposures, uptake rate was assumed to be the product of feeding ration and assimilation efficiency. Separate estimates of Se depuration rates were made from log-linear regressions of bluegill Se concentration vs. time during the depuration periods. Bioconcentration factors (BCF) and food-chain concentration factors (CF) were estimated from ratios of uptake rates to depuration rates or, if no models were produced, from ratios of organism Se concentrations to Se concentrations in water or food. Equilibrium Se concentrations in bluegill were estimated from BCFs and CFs for algae, daphnids, and bluegill, assuming additive Se uptake from aqueous and food-chain exposures (Spacie and Hamelink 1985). This model was also used to estimate bluegill Se bioaccumulation from a mixture of Se species, assuming additive Se uptake from different Se species.

Principal Findings

Distribution and bioaccumulation of selenium in aquatic microcosms: Dissolved Se concentrations in microcosms were affected by sediment type and Se speciation. Decreases in dissolved ^{75}Se concentrations were initially rapid, then became more gradual (Figure 1A). After the first three days, dissolved ^{75}Se decreased more rapidly in microcosms with Volta sediments than in microcosms with San Joaquin sediments. Concentrations of dissolved ^{75}Se were significantly higher in the San Joaquin sediment treatment during the final 14 d of the study. The fine-textured, highly organic Volta sediment accumulated significantly more ^{75}Se than did the San Joaquin sediment (Table 1). Dissolved ^{75}Se activity did not differ significantly between the high- and low-sulfate treatments, but sediment accumulated significantly more ^{75}Se in the low-sulfate treatment. Differences in sediment ^{75}Se accumulation between sulfate treatments (3%, not statistically significant) were much less than differences between sediment treatments (30%, statistically significant).

Dissolved selenomethionine residues decreased more rapidly than residues of selenate and selenite (Figure 2A). Concentrations of ^{75}Se in the selenomethionine treatment decreased rapidly early in the study and remained significantly lower than in the selenate or selenite treatments throughout the study. Concentrations of ^{75}Se in the selenite treatment were significantly lower than in the selenate treatment. The decreasing portion of the selenomethionine-loss curve (days 1-11) and the entire curves for selenate and selenite were approximated by exponential decrease curves, which differed significantly among the three Se species: half-times of dissolved ^{75}Se residues were 7 d for selenomethionine, 20 d for selenite, and 33 d for selenate. Differences in loss rates of dissolved ^{75}Se between selenate and selenite treatments were apparently due to greater sediment accumulation of selenite residues, but sediment Se accumulation was similar in the selenite and selenomethionine treatments (Table 2).

Volatilization rates of Se residues also differed among sediment types and Se species. Volatilization rates of the mixture of Se species declined rapidly in microcosms with both sediment types (Figure 1B). The proportion of added ^{75}Se lost to volatilization was significantly greater in the San Joaquin sediment treatment (Table 1). Volatilization did not differ between low- and high-sulfate treatments. Selenium volatilization was initially rapid in the selenomethionine treatment but decreased throughout the study, whereas volatilization increased gradually from very low initial rates in the selenite and selenate treatments (Figure 2B). The volatilization rate remained significantly greater in the selenomethionine treatment throughout the study, contributing to the greater loss rate for dissolved Se-methionine. Overall, volatilization accounted for 24% of ^{75}Se added as selenomethionine, compared to 1% or less of that added as selenate and selenite (Table 2).

Selenium bioaccumulation differed among biotic components and among treatment groups. Bioaccumulation of ^{75}Se was greater in daphnids than in periphyton or macrophytes (Tables 1 and 2). Bioaccumulation of ^{75}Se by daphnids and periphyton did not differ between sediment or sulfate treatments, but macrophytes accumulated significantly higher ^{75}Se concentrations in microcosms with Volta sediment, perhaps due to greater ^{75}Se accumulation in this sediment. Selenium was bioaccumulated much more readily from selenomethionine than from selenite or selenate, and bioconcentration factors (BCFs) were significantly different among the three Se species. This difference may be due in part to higher BCFs at low dissolved Se concentrations, but organisms accumulated equal or greater Se concentrations from selenomethionine than from selenite or selenate, despite higher dissolved concentrations of the inorganic Se species.

Selenium fate in closed-system microcosms was strongly affected by differences in sediment sorption and bioaccumulation of Se species. Our results suggest that sediment characteristics influence the fate of dissolved Se in shallow aquatic systems with high sediment surface area/volume ratios. This effect was not observed in high-volume in situ lake enclosures (Rudd et al. 1980). The fine texture and high organic content of the Volta sediment apparently favored Se sorption relative to the coarse, inorganic San Joaquin sediment. Selenium uptake by sediment-dwelling organisms and sedimentation of Se-enriched detritus, both favored by higher nutrient levels, may also have contributed to Se accumulation in the Volta sediment. Although increasing sulfate concentrations from 10 to 78 mg liter^{-1} resulted in increased Se bioaccumulation in lake enclosures (Rudd et al. 1980), an increase in sulfate between 80 and 180 mg liter^{-1} did not affect selenium fate or bioaccumulation in our microcosms. Decreased Se uptake would be expected at high sulfate concentrations due to sulfate:selenate antagonism (Shrift 1973), but this effect could have been masked by the low proportion of selenate to total ^{75}Se activity in our microcosms.

Selenate and selenite were more persistent than selenomethionine in the water column of microcosms, but were less readily accumulated by aquatic organisms. Loss rates of dissolved Se species from water increased in the order: selenate < selenite < selenomethionine. The loss rate of selenite added to microcosms was about twice that reported from a selenite addition to an entire lake (Hesslein et al. 1980), but followed a similar exponential decrease curve. Differences in the loss rates of the three Se species from water were apparently caused by differences in sediment sorption, which was greater for selenite and selenomethionine, and differences in bioaccumulation and volatilization, which were greater for selenomethionine. However, differences in the total amounts of each Se species added to our microcosms ($Se^{+6} > Se^{+4} > Se^{-2}$) may also have contributed to differences in Se loss rates.

Bioconcentration factors and volatilization rates were roughly proportional to loss rates from water. Selenium volatilization, primarily of alkylated selenides, has been linked to Se biotransformation and release by aquatic organisms (Cooke and Bruland 1987, Cutter 1989). Unpublished data from our laboratory support this assertion, as no Se volatilization occurred in sterile microcosms. The lower BCFs and volatilization rates of Se added as selenate and selenite suggest that bioaccumulation and biotransformation of these species are slower than that of selenomethionine. Selenium BCFs in microcosms spiked with selenite were similar to those reported for selenite in other model ecosystems (Nassos et al. 1980), but were greater than those reported from large lake enclosures (Turner and Rudd 1983). Results of our microcosm studies are consistent with observations of increased bioaccumulation and toxicity of selenomethionine relative to selenate and selenite (Kleinow and Brooks 1986, Niimi and LaHam 1976).

The persistence and bioavailability of dissolved Se species added to our microcosms have important implications for the fate and effects of Se in aquatic ecosystems. Differences in sediment affinity of dissolved Se species strongly affected their loss rates from water. However, selenite and selenomethionine were accumulated to higher concentrations in biota than selenate, despite being present at lower concentrations in water. These former species may contribute disproportionately to Se bioaccumulation and toxicity in contaminated waters, due to preferential uptake from water or transfer via food chain organisms.

Aqueous and food-chain bioaccumulation of Se species in a laboratory food chain: Selenium bioconcentration by Chlamydomonas reinhardtii differed strongly among Se species and dose levels (Table 3). No BIOFAC models were produced for Se-methionine and selenate exposures, as no samples were collected during the increasing portion of the Se uptake curves (before 3 hours), but BCFs were estimated from the maximum ratio of algae Se concentrations to dissolved Se concentrations. Algal BCFs were greatest for Se-methionine, followed by selenite and selenate, respectively. Bioconcentration from all three Se species decreased at increased aqueous Se concentrations. Se-methionine BCFs remained higher than selenite or selenate BCFs over the range of concentrations tested, but selenite and selenate BCFs were similar at higher dissolved Se concentrations.

Daphnid Se bioconcentration also differed strongly among Se species and dose rates (Table 4). Se-methionine BCFs were extremely high for daphnids, with BCFs over 350,000 at low dissolved concentrations (< 1 ug Se/l), much greater than those for algae at comparable Se-methionine concentrations. Selenite BCFs were much lower, but were 50-

100% greater than those for comparable selenate exposures. Bioconcentration factors for all three Se species decreased with increases in dissolved Se concentrations, primarily due to decreases in uptake rates. Daphnids accumulated aqueous Se species more slowly and took longer to reach equilibrium Se concentrations, with typical half-times ranging from 9 to 24 hr, compared to $\leq$ 3 hr for algae.

Food-chain transfer of algal Se to daphnids was more strongly affected by dose level than by differences in the aqueous Se species used in the algae-daphnid food chain (Table 4). Uptake and depuration rates from models of food-chain transfer from algae to daphnids did not show clear trends across Se species and dose levels. Daphnids accumulated Se concentrations proportional to Se concentrations in the algae they were fed, regardless of the Se species used to dose the algae (Figure 3). Food-chain concentration factors (CF = ratio of uptake rate to depuration rate or ratio of equilibrium daphnid Se concentration to Se concentration in food) were consistent across Se species, but showed a consistent decreasing trend with increases in algal Se concentrations, from near 1.0 at low doses to less than 0.5 at the highest dose. These results indicate that food-chain Se bioaccumulation alone would not produce biomagnification of Se concentrations from algae to daphnids, except perhaps at very low Se dose levels (i.e., < 1 ug/l).

In both aqueous and food-chain exposures, Se-methionine produced greater Se concentrations in bluegill than exposures using the inorganic Se species (Table 5). Bioaccumulation also differed significantly among food chains based on the "natural" ratio of Se species. Highest bluegill Se concentrations occurred in the food chain based on 100 ug Se/l as selenate, but Se concentrations in food chains dosed with 1 ug Se/l as Se-methionine were significantly greater than in those dosed with 10 ug Se/l as selenite. Within each food-chain study, Se uptake was roughly proportional to daphnid Se concentrations, reflecting differences in bioaccumulation of Se species in the algae-daphnid food-chains. However, food-chain Se uptake varied considerably among studies, due to differences in feeding rations.

Bluegill accumulated greater Se concentrations from food than from water in selenite-based exposures (Table 5). Mean Se concentrations in bluegill from food-chain exposures based on selenite concentrations of 10 and 100 ug Se/l were significantly greater than concentrations from comparable aqueous exposures during the first three weeks of the studies. Selenium concentrations in bluegill tissues in most food-chain exposures remained higher than in corresponding aqueous exposures on day 30, despite increased Se uptake rates in some aqueous exposures after day 21. Increases in Se uptake rates late in aqueous exposures were apparently due to ingestion of Se-containing solids (particulates). Aqueous and food-chain Se bioaccumulation were approximately additive. Selenium concentrations in bluegill from combined exposures averaged 106% of sums of concentrations in aqueous and feeding exposures after 30 days.

Bioconcentration models suggested differences in Se-uptake kinetics among Se species, although not all aqueous exposures produced adequate models (Table 6). Models produced from aqueous Se-methionine exposures indicated very high Se-uptake rates and bioconcentration factors, although depuration rates differed widely between models from the two exposures. The model from Study 2 (18-day exposure) suggests that bluegill equilibrated rapidly with aqueous Se-methionine (half-time = 3.3 days), but the model from Study 1 (37-day exposure) suggested a slower depuration rate and more gradual equilibration of bluegill Se concentrations (half-time = 20 days). The BCF (4,600) produced from the model of Study 2 was consistent with BCFs estimated from bluegill and dissolved Se concentrations in both studies (4,900 and 4,500 in Studies 1 and 2, respectively). Bluegill accumulated Se slowly from dissolved selenite, and Se uptake was approximately linear, resulting in inadequate or inconsistent estimates of depuration rates. Because of the slow Se uptake rates, bluegill Se concentrations in longer selenite exposures were also sensitive to ingestion of Se-contaminated solids. Estimates of selenite and selenate BCFs ranged from 13 to 100 (mean BCF = 50), and ratios of bluegill to water Se concentrations approached this range in 30- to 37-day exposures. Selenite and selenate BCFs were lower at higher dissolved Se concentrations. Bluegill BCFs for all three Se species were lower than those measured for algae or daphnids.

Models of foodborne-Se transfer from daphnids to bluegill were developed only for exposures with both uptake and depuration phases (Table 6). Uptake curves from the comparisons of Se species (Studies 1 and 2) were all strongly linear and did not produce good estimates of depuration rates. However, the slopes of these curves were linearly related to Se dose levels, regardless of which Se species was used to dose the food chains (Figure 4). Models produced in selenite exposures with both uptake and depuration data (Studies 3 and 4) differed between dose levels. Assimilation efficiencies were higher at the low dose levels in both studies. Food-chain concentration factors differed between the two studies, ranging from 0.8 to 1.6, but did not differ between dose levels.

Depuration rates of bluegill Se residues accumulated from different Se species and exposures routes were similar (Table 6). Depuration rates estimated directly from depuration curves in Study 4 were similar for aqueous, food, and combined selenite exposures, averaging 0.035 day^{-1} (half-time = 20 days). This depuration rate suggests that bluegill would require an exposure period of 65 days to reach 90% of equilibrium Se concentrations, which is consistent with the failure of bluegill to reach steady-state Se concentrations in our 18- to 37-day exposures. Depuration rates estmated from BIOFAC aqueous bioconcentration models were more variable. In aqueous selenite exposures, depuration rates from models based on uptake curves corresponded to half-times of 4-10 days, whereas the slower depuration rates from models of combined uptake and depuration curves suggested half-times of 26-50 days. The only selenate bioconcentration model suggested a very slow depuration rate (half-time = 70 days). Models produced from the 18-day Se-methionine exposure (Study 2) suggested a very rapid equilibration with dissolved Se-methionine (half-time = 3 days), but the longer exposure (Study 1) produced a depuration rate similar to those estimated for selenite-based exposures. This suggests that Se-methionine residues are highly labile when first accumulated and become more tightly bound over time.

Equilibrium bluegill Se concentrations under various exposure regimes were estimated based on results of Se exposures with algae, daphnids, and bluegill. Bioconcentration of Se species was extrapolated from observed BCFs, and two models were used to estimate the range of bluegill food-chain Se bioaccumulation. The "conservative" model assumed that only food-chain transfer contributed to daphnid Se concentrations, an assumption which corresponds directly to the water-algae-daphnid-bluegill exposures performed in the laboratory. The "worst-case" model assumed that daphnid-Se uptake from aqueous and food-chain exposures were additive, and that all daphnid-Se residues were equally available to bluegill. A maintenance ration (0.5 % day^{-1}) and a single depuration rate (0.035 day^{-1}) were assumed for all bluegill food-chain models. Assimilation efficiency was assumed to be unaffected by Se speciation and to decrease with increases in daphnid Se concentration.

Both total Se concentrations in bluegill and the contributions of aqueous and food-chain exposures differed among models of exposures to different Se species (Figure 5).

Both conservative and worst-case models based on Se-methionine exposures predicted bluegill Se concentrations 10-30 times greater than than those from comparable exposures with selenite and selenate. Greater bluegill Se concentrations were predicted from selenite than from selenate at low dose levels, but both species produced similar Se concentrations at higher dose levels. Food-chain transfer was the primary route of bluegill Se bioaccumulation from all three Se species. The contribution of food-chain bioaccumulation to bluegill Se concentrations was greatest at lower nominal Se concentrations, due to more efficient assimilation of food-borne Se at lower dose levels. Food-chain transfer was consistently more important for selenite (80-98% of total Se residues) than for selenate (51-88%). Food-chain contributions in Se-methionine models ranged from 30% at the highest dose of the conservative model to 98% at the low dose level of the worst-case model. This wide range reflects the potentially large contribution of food-chain Se transfer in the worst-case model, due to the extremely high bioconcentration of Se-methionine by daphnids. Models simulating a 1:10:100 mixture of Se-methionine, selenite, and selenate predicted substantially greater bluegill Se concentrations than those produced by comparable concentrations of selenite or selenate. Se-methionine contributed 30-75% of the bluegill Se concentrations predicted from exposures to this mixture.

Differential bioconcentration of aqueous Se species was observed in aqueous exposures with all three study taxa, but Se residues accumulated from different aqueous Se species were equally assimilable by aquatic biota. Selenium bioconcentration was greatest from Se-methionine by a wide margin, followed by selenite and selenate, respectively. Our results are similar to those of Sandholm et al. (1973), who reported differences in Se bioconcentration of Se-methionine and selenite by algae (Scenedesmus dimorphus) and daphnids (D. pulex) in microcosms, but found that zooplankton Se residues accumulated from different Se species were equally available to fish. The same trends among Se species observed in bioconcentration studies have also been reported in toxicity studies with blue-green algae (*Anacysitis nidualans* and *Anabaena variabilis*; Kumar and Prakash 1971), daphnids and midge larvae (*D. magna* and *Chironomus riparius*; Ingersoll et al. 1990) and zebrafish (*Brachydanio rerio*; Niimi and LaHam 1976). Kleinow and Brooks (1986) reported more rapid uptake of Se-methionine than of selenite or selenate, when administered directly into the gastrointestinal tract of fathead minnows, but suggested that Se residues from different Se species converged over time to a common metabolic pool. The similarity of bluegill Se depuration rates from aqueous, food-chain, and combined Se exposures suggests that Se residues in bluegill also converge to a common metabolic pool. The hypothesis that aqueous Se species (primarily inorganics) are converted to Se-amino acids and Se-proteins in aquatic organisms has been supported by preliminary results of microcosm studies at our laboratory, and by Knight (1988).

Results of our aqueous and food-chain Se exposures are consistent with observations that Se toxicity is strongly linked to Se bioaccumulation. The highest Se bioconcentration observed in our exposures, in daphnid Se-methionine exposures, was accompanied by substantial mortality, and Ingersoll et al. (1990) also reported acute toxicity of Se-methionine to *D. magna* at similar concentrations (5-10 ug Se/l). Bluegill and other centrarchids have proven to be relatively sensitive to Se bioaccumulation and toxicity in labratory and field studies, and our estimates for bluegill BCFs for selenite and selenate (average BCF = 50) are higher than those reported for less-sensitive fish taxa in comparable exposures: fathead minnows BCFs ranged from 12-29 for selenite (Adams 1976) and from 22-27 for selenate (Bertram and Brooks 1986); and rainbow trout, *Salmo gairdneri*, had a BCF of 8.3 for selenite (Hodson et al. 1980). Lemly (1982) reported even higher BCFs (300-450) for larger bluegill and largemouth bass (Micropterus salmoides) in selenite exposures. Results of our bluegill feeding exposures are consistent with data from Cumbie and Van Horn (1978) which suggest that bluegill in Se-contaminated Belews Lake, North Carolina, accumulated Se concentrations greater than those in available planktonic food base. Our estimates of daphnid-bluegill food-chain CFs (0.8-1.6) were also greater than those reported for fathead minnows fed via similar laboratory food chains (CFs = 0.21-0.36; Bertram and Brooks 1986)

Our models of Se bioaccumulation suggest that Se transfer via aquatic food chains, possibly including contributions from organoselenium species such as Se-methionine, contributes significantly to accumulation of toxic concentrations of Se residues in bluegill. Several researchers have associated reproductive toxicity in bluegill and other fish with tissue Se concentrations of 5-15 ug/g (Cumbie and Van Horn 1978, Lemly 1985, Gillespie and Baumann 1986, Coughlan and Velte 1989, Hamilton et al. 1989). Our model indicates that aqueous bioconcentration from selenate or selenite concentrations up to 100 ug Se/l would not produce bluegill Se concentrations over 5 ug/g, whereas combined aqueous and food-chain exposures to selenite or selenate at 10 ug Se/l would produce bluegill Se concentrations in the 5-15 ug Se/g range. In contrast, models based on Se-methionine exposures produced equal or greater bluegill Se concentrations from much lower dissolved Se concentrations (1-10 ug Se/l in aqueous exposures and 0.1-1 ug/l in combined exposures). A hypothetical "natural" mixture of Se-methionine, selenite, and selenate (1:10:100 ratio) would produce potentially toxic Se concentrations in bluegills from aqueous concentrations of 10 ug Se/l or less. Se-methionine contributed a large portion of the total bioaccumulation from this mixture, and low concentrations of organoselenium compounds may also contribute substantially to bioaccumulation and toxicity in contaminated natural systems.

Summary

Closed-system microcosms were used to study factors affecting the fate of selenium (Se) in aquatic systems. The distribution and bioaccumulation of Se in microcosms varied among sediment types and Se species. A mixture of dissolved ^{75}Se species (selenate, selenite, and selenomethionine) was sorbed more rapidly to fine-textured, highly organic pond sediments than to sandy riverine sediments. Sulfate concentrations did not affect the distribution and bioaccumulation of the ^{75}Se mixtures over the range 80-180 mg SO_4 liter^{-1}. When each Se species was labeled separately, selenomethionine was lost from the water column more rapidly than selenate or selenite. Selenium lost from the water column accumulated primarily in sediments, but volatilization was also an important pathway for loss of Se added as selenomethionine. Loss rates of dissolved Se residues were more rapid than rates reported from mesocosm and field studies, suggesting that sediment:water interactions are more important in microcosms than in larger test systems. Daphnids accumulated highest concentrations of Se, followed by periphyton and macrophytes. Selenium added as selenomethionine was bioaccumulated preferentially compared to that added as selenite or selenate, suggesting that organoselenium compounds may contribute disproportionately to Se bioaccumulation and toxicity in aquatic organisms.

Selenium bioconcentration and food-chain bioaccumulation from three Se species (selenate, selenite, and selenomethionine) were determined in a laboratory food chain consisting of algae (Chlamydomonas reinhardtii), daphnids (Daphnia magna), and fish (bluegill, Lepomis macrochirus). Selenium concentrations in water, food, and test organisms were monitored with ^{75}Se-radiolabeled compounds, and Se uptake and depuration were described with two-compartment kinetic models. Selenium bioconcentration differed among aqueous Se species and among test organisms. All organisms concentrated Se more strongly from selenomethionine than from either inorganic Se species, and algae and daphnids concentrated Se more strongly from selenite than selenate. In general, bioconcentration of aqueous Se species was greatest in algae and successively less in daphnids and bluegill, respectively. Bioconcentration factors generally decreased with increases in aqueous Se concentrations. Food-chain transfer from algae to daphnids to bluegill was not affected by the aqueous Se species used to dose the food chains, but differential bioconcentration of aqueous Se species by algae and daphnids affected the amount of food-borne Se available to bluegill. Concentration factors (CFs), ratios of Se concentrations in consumers to concentrations in their food indicated that daphnids and bluegill accumulated Se concentrations similar to those in food organisms. Daphnid CFs decreased from near 1.0 to less than 0.5 with increases in the dose of Se-contaminated algae they recieved. Bluegill CFs in food-chains dosed with selenite ranged from 0.8 to 1.6. Aqueous and food-chain Se uptake by bluegill were approximately additive, and Se depuration rates were approximately equal in bluegill from aqueous, food-chain, and combined (food + water) exposures. The average depuration rate in bluegill exposures corresponded to a biological half-time for Se of 20 days. Bioaccumulation models suggested that food-chain transfer is the predominant route of bluegill Se bioaccumulation from all three Se species and that low environmental concentrations of Se-methionine or other organoselenium compounds may contribute disproportionately to Se bioaccumulation and toxicity in aquatic organisms.

Research Needs

Occurrence and bioavailability of organoselenium compounds - Our research found that a single organoselenium compound, Se-methionine, is more biologically available than the predominant inorganic Se species and could contribute substantially to Se bioaccumulation in aquatic consumers, even if present at very low concentrations in the environment. Studies of Se speciation in natural aquatic ecosystems (Cooke and Bruland 1987, Cutter 1989) have found that organoselenium compounds are present in water and that their concentrations vary both spatially and seasonally. However, the specific compounds present, the mechanisms for their formation, and their bioavailability to aquatic biota have not been adequately studied. We have attempted to develop methodologies for separating and quantifying fractions of dissolved organoselenium compounds, especially the Se-amino fraction, in water from experimental microcosms. This research, if completed, could describe the formation and release of organoselenium compounds and provide methodologies which could be applied to monitoring these compounds in the environment. Another essential component of such research would be qualitative identification of compounds in various dissolved Se fractions.

Aqueous and food-chain Se bioaccumulation - Results of our bioaccumulation studies, combined with Se residue data from toxicity tests conducted at our laboratory have documented bluegill Se bioaccumulation from organic and inorganic Se species, via aqueous and food-chain exposure routes. However, the short duration and limited dose range of our bluegill Se exposures, and the limitations of sampling schedules in the toxicity studies have resulted in some uncertainty regarding Se uptake kinetics under certain exposure conditions. These problems do not affect the clear trends in bluegill Se bioaccumulation among Se species and exposure routes, but they reduce the predictive ability of the bioaccumulation models presented in this report. A more careful examination of the bioaccumulation data from all these studies may allow a more authoritative description of Se residue dynamics in bluegill.

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Table 1. Distribution and bioaccumulation of ^{75}Se after 28 days in Fate Study 1.[a]

Variable	Sample	San Joaquin sediment		Volta sediment	
	Type	Low-SO4 (N=2)	High-SO4 (N=3)	Low-SO_4 (N=3)	High-SO_4 (N=2)
Se Distribution (%)	Volatile[b]	7.9 ± 1.8	7.2 ± 1.2	4.6 ± 0.3	3.0 ± 0.5
	Dissolved[b]	48.9 ± 1.7	54.5 ± 2.0	29.6 ± 0.2	28.2 ± 1.7
	Suspended[b]	3.4 ± 0.3	3.3 ± 0.2	1.7 ± 0.04	1.7 ± 0.1
	Sediment[b,c]	35.3 ± 2.3	31.4 ± 0.8	62.4 ± 0.2	61.1 ± 1.9
	Biota	4.5 ± 1.8	3.6 ± 1.2	1.6 ± 0.2	5.9 ± 0.5
Se Bioaccumulation (μCi/g)	Macrophytes[b]	0.06[d]	0.05 ± 0.03	0.39 ± 0.02	0.22 ± 0.13
	Periphyton	2.49 ± 0.12	1.10 ± 0.19	0.74 ± 0.08	1.26 ± 0.08
	Zooplankton	5.04[d]	2.76 ± 0.52	1.20 ± 0.78[e]	2.95 ± 0.20

[a] Means by treatment group ± standard error.

[b,c] Significant difference between (b) sediment types and/or (c) sulfate levels

[d] N=1 (sample recovered from only 1 microcosm).

[e] N=2.

Table 2. Distribution and bioaccumulation of ^{75}Se after 28 days in Fate Study 2.[a]

Variable	Sample type	Selenate	Selenite	Se-methionine
Selenium Distribution (%)				
	Volatiles[b]	0.5 ± 0.03	1.1 ± 0.2	24.0 ± 1.7
	Dissolved[b]	50.0 ± 1.6	32.0 ± 2.5	13.9 ± 1.0
	Suspended	2.2 ± 0.1	2.9 ± 0.4	3.2 ± 0.5
	Sediment[b]	46.8 ± 1.5	62.7 ± 2.7	55.7 ± 1.1
	Biota[b]	0.5 ± 0.1	1.2 ± 0.4	3.2 ± 0.5
Selenium Concentration				
	Water (µg/liter)	19.0 ± 0.4	2.5 ± 0.3	0.3 ± 0.01
	Macrophytes (µg/g)	1.3 ± 0.6	1.0 ± 0.6[c]	0.9 ± 0.2
	Periphyton (µg/g)	2.7 ± 0.4	1.8 ± 0.3	4.3 ± 1.7
	Zooplankton (µg/g)	6.7 ± 0.9	2.5 ± 1.3	7.5 ± 2.2
Bioconcentration factor				
	Macrophytes[b]	72 ± 32	363 + 147[c]	3266 ± 535
	Periphyton[b]	141 ± 22	755 ± 138	16836 ± 6738
	Zooplankton[b]	351 ± 42	1087 ± 611	23870 ± 9369

[a] Means ± standard error; N=3 unless otherwise indicated.

[b] Significant difference among treatments and among all treatment means.

[c] N=2.

Table 3. Algal Se bioconcentration from three Se species. Uptake and depuration rates and BCFs were derived from BIOFAC models, except BCFs in parentheses, which were estimated from ratios of algae Se concentrations to dissolved Se concentrations.

Se Species	Nominal Se Conc. (ug/l)	k_u (hr^{-1})	k_d (hr^{-1})	BCF
Se-methionine	0.1	(no model)		(54,400)
	1.0	(no model)		(23,100)
	10.0	(no model)		(7,200)
Selenite	1.0	438	0.20	2,136
	10	516	0.32	1,637
	100	121	0.26	472
Selenate	10	(no model)		(590)
	100	(no model)		(550)
	1000	(no model)		(420)

Table 4. BIOFAC models of daphnid Se bioconcentration and food-chain Se bioaccumulation from three Se species.

Selenium Species	Nominal Se Conc. (ug/l)	Aqueous Exposures k_u (hr^{-1})	k_d (hr^{-1})	BCF	Food-chain Exposures k_u (d^{-1})	k_d (d^{-1})	CF
Se-methionine	0.1	17,274	0.047	369,673		(no model)	
	1.0	7,151	0.029	244,216	0.399	0.389	0.819
	10	1,721	0.062	27,633	0.770	1.10	0.699
Selenite	1.0	234	0.075	3,129	0.252	0.384	0.657
	10	23	0.041	572		(no model)	
	100	12	0.057	217	0.143	0.251	0.570
Selenate	10	49	0.18	273	0.371	0.469	0.791
	100	7.0	0.049	145	0.448	0.725	0.618
	1000	5.0	0.070	72	0.107	0.229	0.469

Table 5. Selenium bioaccumulation by bluegill (*Lepomis macrochirus*) during aqueous and food-chain exposures based on three Se species. Water and daphnid Se concentrations are means over the course of each study; bluegill dry weights and Se concentrations are means (SD = standard deviation) at the end of the exposure period (initial dry weights are in parentheses). Within each group of three treatments, bluegill Se concentrations followed by the same letter are not significantly different.

Selenium Species	Exposure route/ Dose level	Water Se conc. (ug/l)	Daphnid Se conc. (ug/g)	Feeding Ration (%/day)	Bluegill Dry Wt. (g)	Bluegill Se conc. (ug/g ± SD)
Study 1 (37 days)						
					(0.039)	
Se-methionine	Water/10 ppb	8.7	0.11	1.1	0.024	42.7 ± 12.4 a
Selenite	Water/10 ppb	10.6	0.11	1.2	0.019	1.17 ± 0.47 b
Selenate	Water/10 ppb	10.9	0.11	1.2	0.028	1.02 ± 0.63 b
Se-methionine	Food/10 ppb	0.005	5.5	0.85	0.029	0.70 ± 0.15 a
Selenite	Food/10 ppb	0.002	2.7	0.85	0.032	0.23 ± 0.03 b
Selenate	Food/10 ppb	0.003	1.4	1.29	0.030	0.18 ± 0.07 b
Study 2 (18 days)						
					(0.025)	
Se-methionine	Water/1 ppb	0.50	--	--	0.032	2.27 ± 0.13 a
Selenite	Water/10 ppb	9.3	--	--	0.027	0.25 ± 0.04 c
Selenate	Water/100 ppb	96	--	--	0.029	1.77 ± 0.14 b
Se-methionine	Food/1 ppb	0.01	1.9	5.9	0.034	0.71 ± 0.15 b
Selenite	Food/10 ppb	0.008	1.3	5.6	0.034	0.42 ± 0.09 c
Selenate	Food/100 ppb	0.009	3.8	8.2	0.032	1.70 ± 0.15 a

Table 5 (continued).

Selenium Species	Exposure route/ Dose level	Water Se conc. (ug/l)	Daphnid Se conc. (ug/g)	Feeding Ration (%/day)	Bluegill Dry Wt. (g)	Bluegill Se conc. (ug/g ± SD)
Study 3 (30 days)						
					(0.080)	
Selenite	Water/10 ppb	9.24	0.30	1.25	0.062	0.34 ± 0.23 a
Selenite	Food/10 ppb	0.01	3.68	1.29	0.079	0.60 ± 0.06 a
Selenite	Combined/10 ppb	9.00	3.68	1.40	0.065	0.82 ± 0.16 a
Selenite	Water/100 ppb	94	0.30	1.21	0.070	1.77 ± 0.74 b
Selenite	Food/100 ppb	0.00	23.3	1.13	0.090	3.23 ± 0.16 a
Selenite	Combined/100 ppb	93	23.3	1.36	0.056	5.53 ± 1.70 a
Study 4 (30 days)						
					(0.046)	
Selenite	Water/10 ppb	10.3	0.12	1.6	0.032	0.84 ± 0.24 a
Selenite	Food/10 ppb	0.005	3.7	1.4	0.027	0.56 ± 0.06 b
Selenite	Combined/10 ppb	10.3	3.7	1.5	0.025	1.18 ± 0.36 a
Selenite	Water/100 ppb	102	1.2	1.3	0.037	3.42 ± 1.66 b
Selenite	Food/100 ppb	0.004	33	1.4	0.033	4.29 ± 0.39 b
Selenite	Combined/100 ppb	102	33	1.5	0.033	8.63 ± 1.30 a

Table 6. BIOFAC models of bluegill Se bioconcentration and bioaccumulation from three Se species. Models derived from combined uptake and depuration curves are indicated by asterisks; depuration rates estimated directly from depuration curves are in parentheses.

Selenium Species	(Study Number)	Nominal Se Conc. (ug/1)	Aqueous Exposures			Food-chain Exposures			
			k_u (d^{-1})	k_d (d^{-1})	BCF	k_u (d^{-1})	alpha	k_d (d^{-1})	CF
Se-methionine	(2)	1.0	958	0.21	4,647	(no model)			
	(1)	10	243	0.035	6,976	(no model)			
Selenite	(1)	10	(no model)			(no model)			
	(2)	10	2.5	0.068	37	(no model)			
	(3)	10	(no model)			0.0074	0.53	0.018	1.50*
	(4)	10	2.8 2.6	0.098 0.027 (0.041)	28 97*	0.0072	0.52	0.031 (0.031)	0.83*
	(3)	100	(no model)			0.0050	0.37	0.012	1.61*
	(4)	100	0.89 0.80	0.067 0.014 (0.031)	13 58*	0.0061	0.44	0.025 (0.038)	0.87*
Selenate	(1)	10	(no model)			(no model)			
	(2)	100	1.1	0.010	66	(no model)			

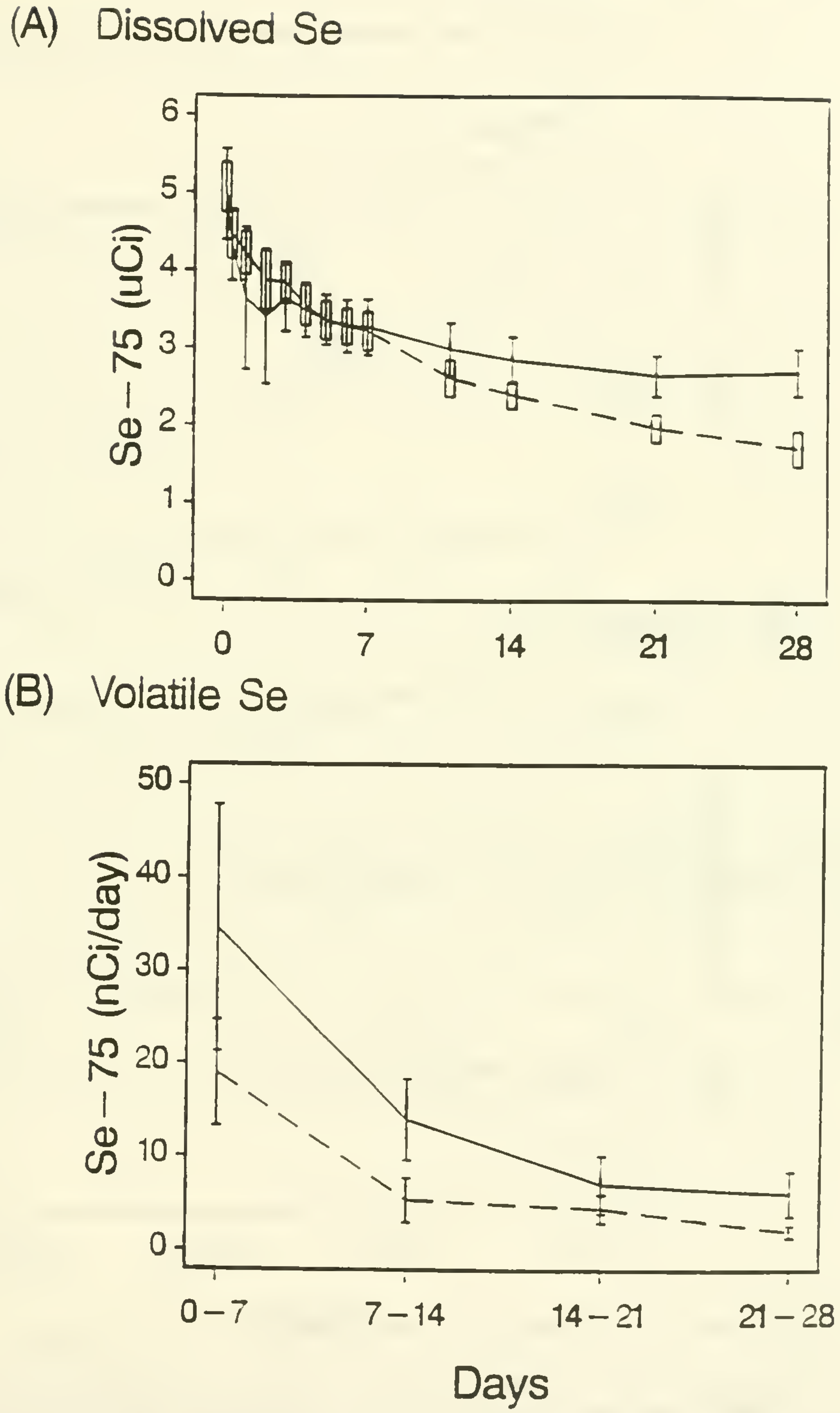
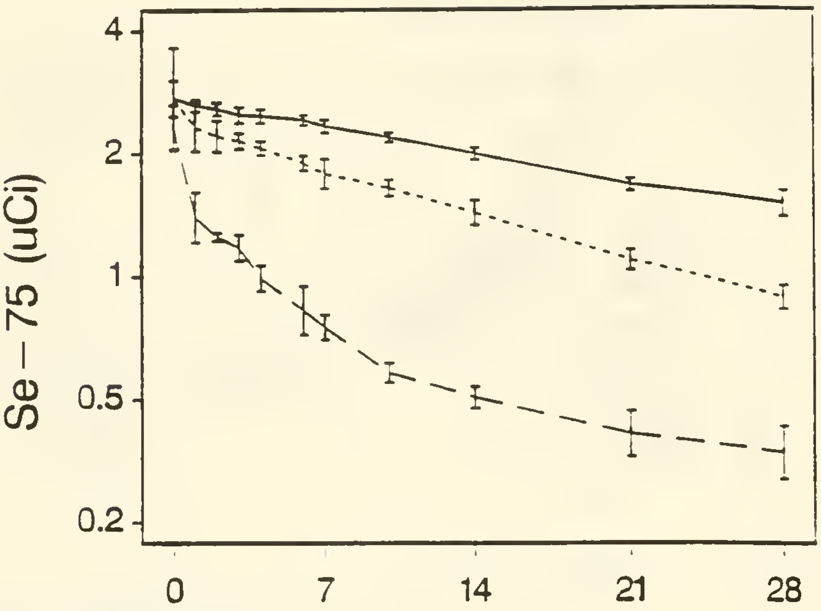
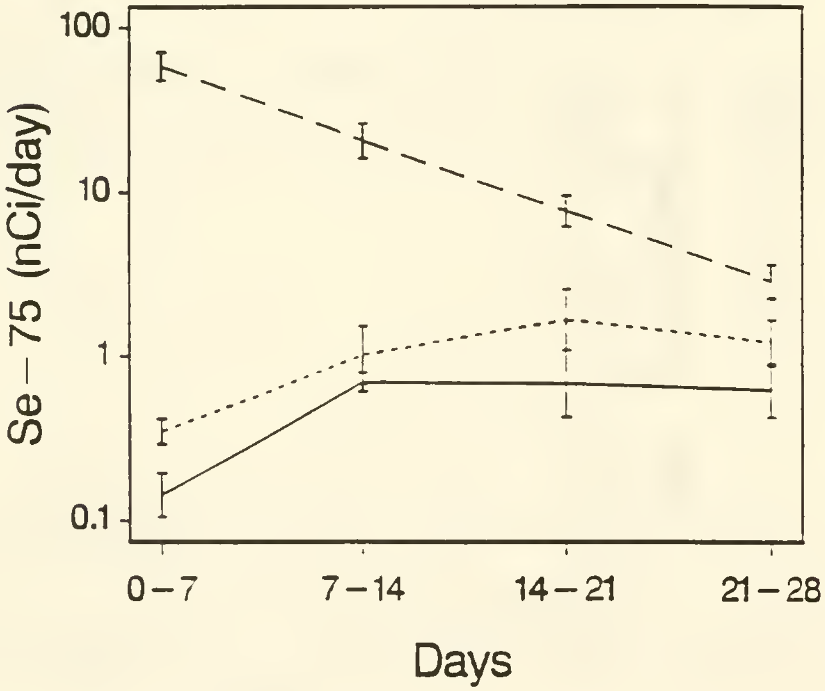


Fig. 1. Dissolved and volatile Se in microcosms during Fate Study 1. Mean ^{75}Se activities ± standard error. Solid line=San Joaquin sediment; dashed line=Volta sediment. N=5 microcosms per sediment type. A. Dissolved Se (μCi/microcosm). B. Volatile Se (nCi/microcosm/day).



(A) Dissolved Se



(B) Volatile Se

Fig. 2. Dissolved and volatile Se in microcosms during Fate Study 2. Mean ^{75}Se activities ± standard error, plotted on logarithmic scale. Solid line=selenate, dotted line=selenite, dashed line=Se-methionine. N=3 microcosms per Se species. A. Dissolved Se (μCi/microcosm). B. Volatile Se (nCi/microcosm/day).

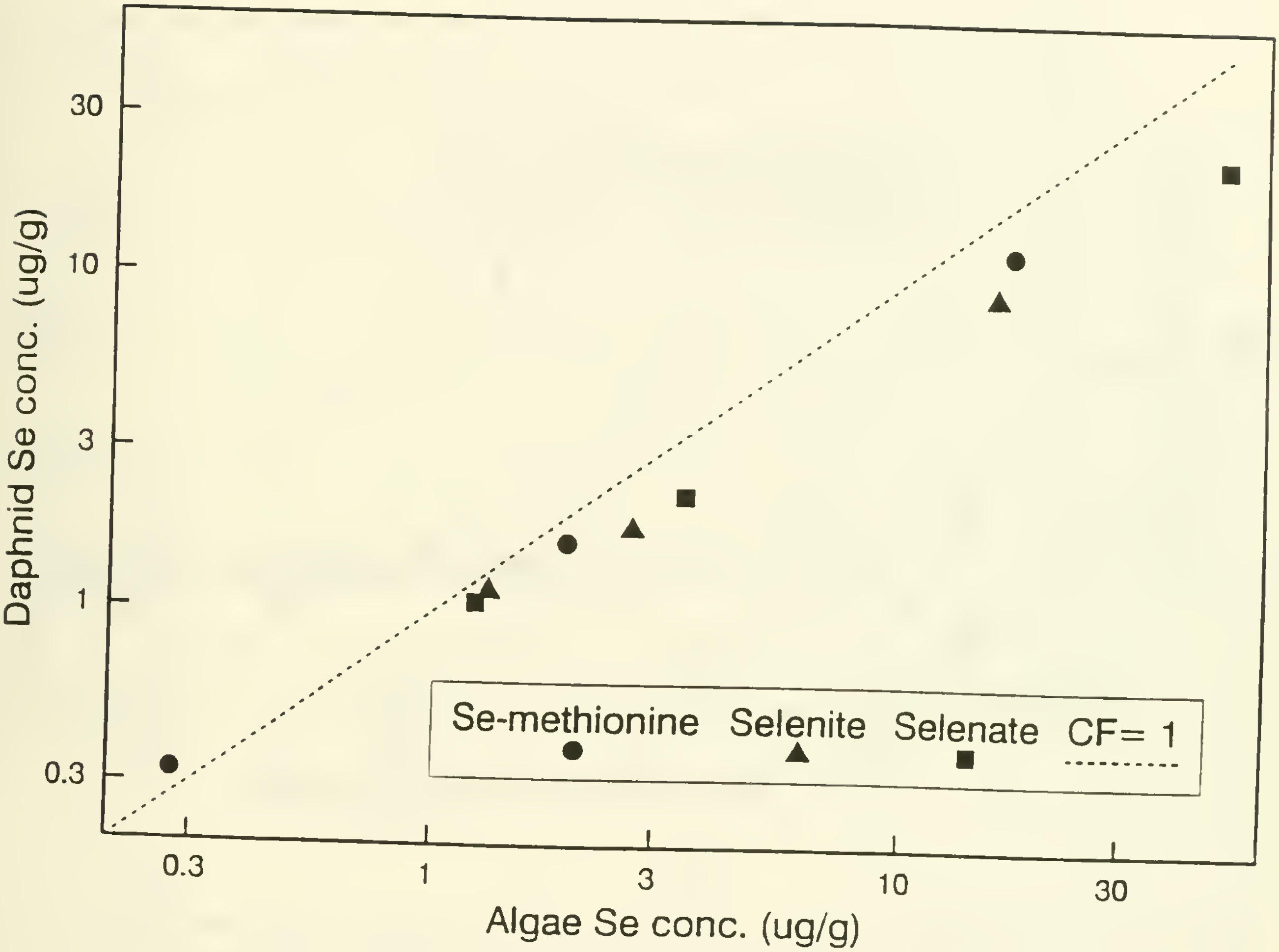


Figure 3. Log-log plot of steady-state daphnid Se concentrations versus average Se concentrations in algae during daphnid feeding exposures. Symbols represent exposures based on different Se species, as indicated in legend; reference line indicates concentration factor = 1 (i.e. daphnid Se concentration = algae Se concentration).

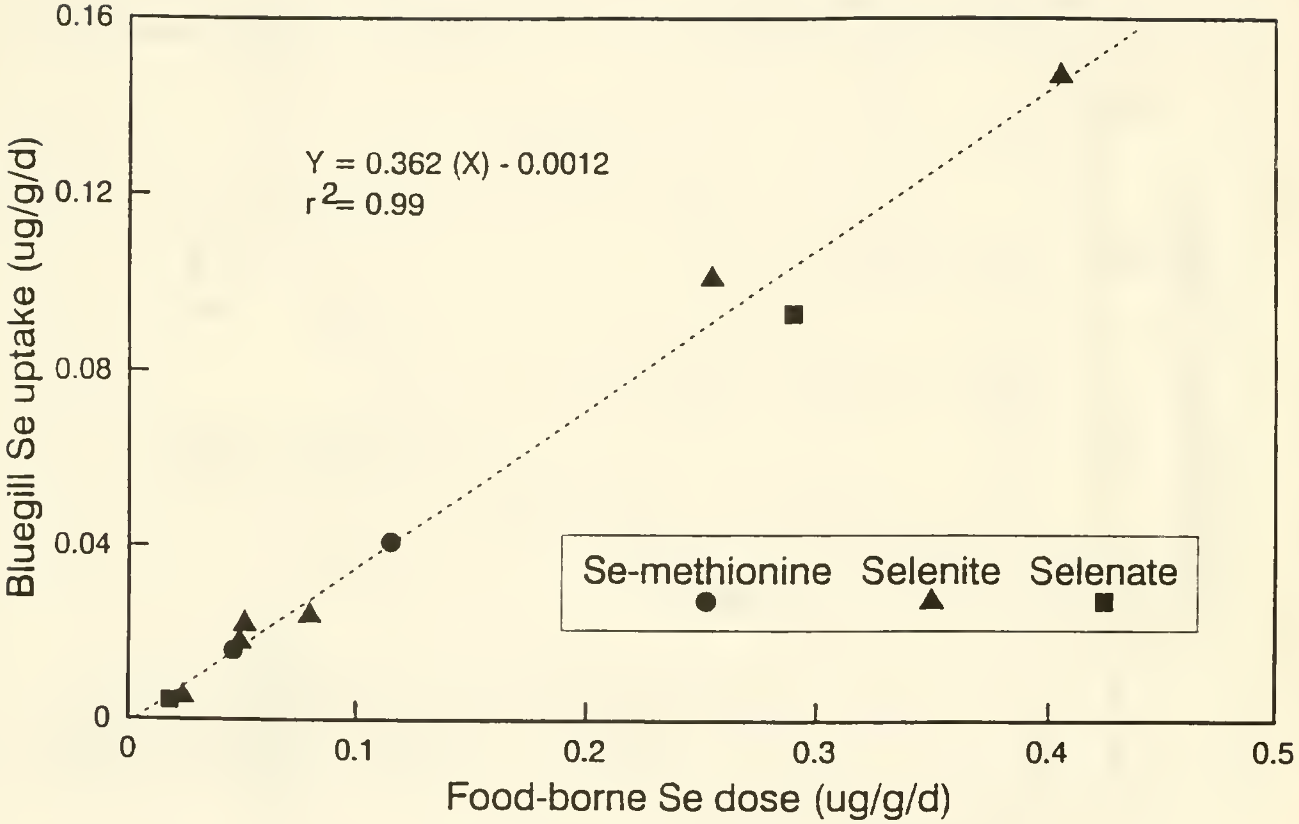


Figure 4. Plot of slopes of bluegill Se uptake curves versus dose rates of Se-contaminated daphnids from bluegill feeding exposures. Symbols represent exposures based on different Se species, as indicated in legend; reference line and equation refer to linear regression of all data points.

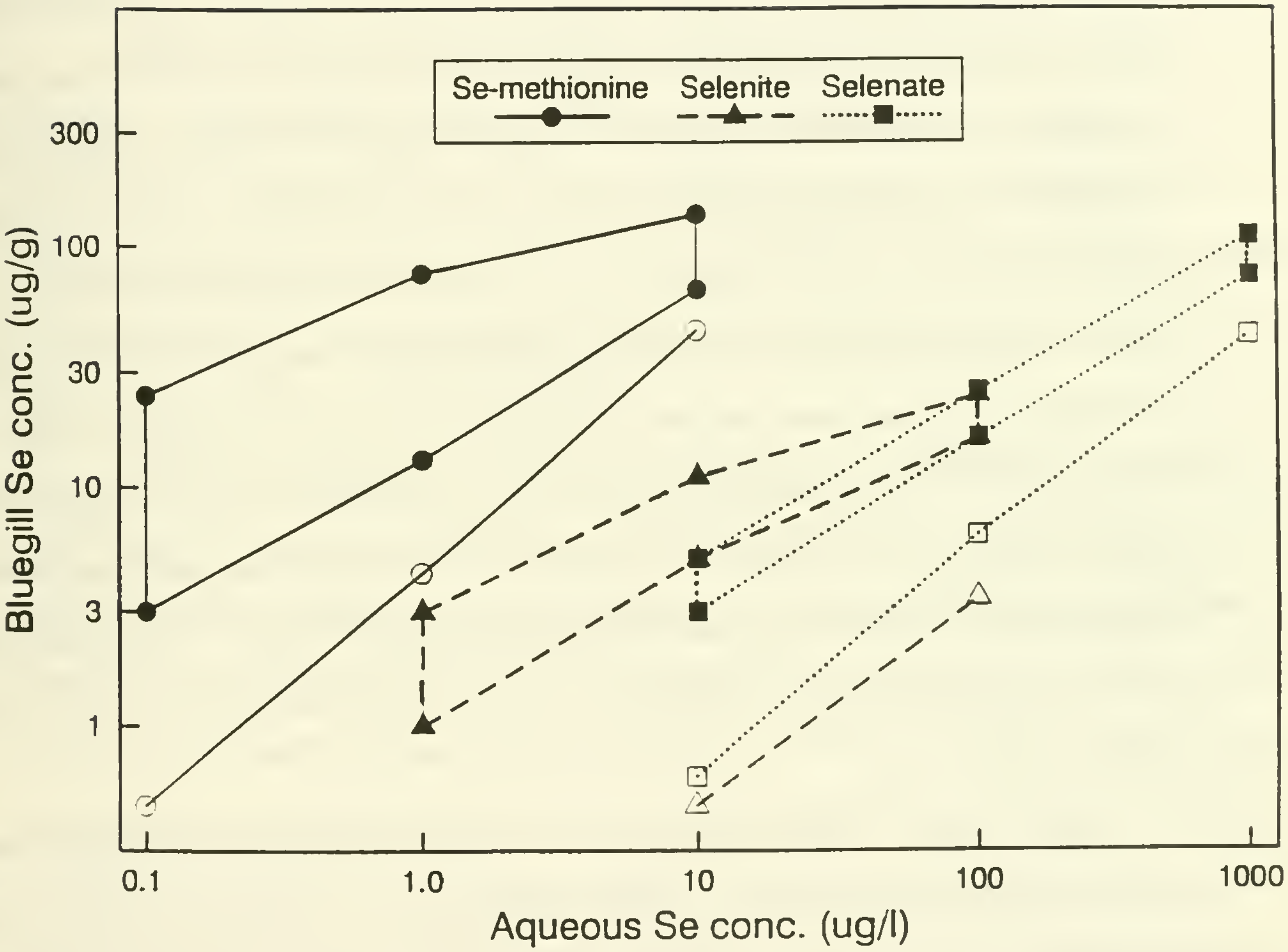


Figure 5. Equilibrium Se concentrations in bluegill (L. macrochirus) from models of aqueous and combined (aqueous and food-chain) exposures to Se species. Symbols represent Se species, as indicated in legend: hollow symbols represents models of aqueous Se bioconcentration; solid symbols represent models of combined aqueous and food-chain Se bioaccumulation.

BIOACCUMULATION OF CONTAMINANTS IN SAN LUIS DRAIN WATER: FIELD BIOACCUMULATION STUDIES

Introduction

Agricultural drainwater--a combination of surface return flows (tailwater) and subsurface (tile) drainage--from about 31,000 ha of irrigated croplands north of Mendota in the San Joaquin Valley, California, is transported through the Grassland Water District (Grasslands) in an interconnected system of canals, ditches, and sloughs for disposal in the San Joaquin River (Presser and Barnes, 1985). In 1984, elevated concentrations of selenium were found in fish from several locations in the Grasslands; these concentrations--up to 23 μg/g dry weight in whole green sunfish (Lepomis cyanellus)--were nearly twice as high as the minimum threshold concentration (12 μg/g) that can elicit reproductive failure and other problems in selenium-sensitive species such as centrarchids (Lemly and Smith 1987). Expanded field surveys in 1985-86 verified that fish from the Grasslands contained excessive concentrations of selenium, and also demonstrated that fish from the San Joaquin River were accumulating elevated concentrations of this element (Saiki and Lowe, 1987; Saiki and May, 1988).

Other elements of concern in agricultural drainwater from the San Joaquin Valley include arsenic, boron, chromium, mercury, and molybdenum (Presser and Barnes 1985). All of these elements are toxic to aquatic organisms when present at concentrations above which the organisms can safely metabolize and excrete. Some of these elements (e.g., selenium) accumulate in fish primarily through exposure to contaminated foods.

The primary objective of this field survey was to determine if trace elements had accumulated to potentially toxic concentrations in aquatic food chains (including fish) that received markedly different exposures to agricultural drainwater. A secondary objective was to verify that the forage groups (taxa) selected for analysis of trace elements were indeed key components of the aquatic food chains of fish.

Methods

Samples of water, sediment, particulate organic detritus, filamentous algae, net plankton, aquatic macroinvertebrates (chironomids, amphipods, and crayfish), and fish (mosquitofish, *Gambusia affinis*; bluegills, *Lepomis macrochirus*; and largemouth bass, *Micropterus salmoides*) were collected in the spring (February-May) and fall (September-December) of 1987. The spring and fall collections represented periods of high and low flows, respectively, in the San Joaquin River.

Collections were made from a total of eight sites: three east-side tributaries of the San Joaquin River whose flows consist mostly of rainfall and snowmelt from the Sierra Nevada and some tailwater but no tile drainage (i.e., the Merced, Tuolumne, and Stanislaus rivers); two west-side tributaries of the San Joaquin River whose flows are based almost entirely on a mixture of tailwater and tile drainage (i.e., Salt and Mud (north) sloughs, both located in the Grasslands); and three sites on the San Joaquin River (Figure 1). Sites on the San Joaquin River were situated as follows: the first site (at Lander Avenue) was located upstream from the west-side tributaries contaminated by tile drainwater; the second site (above Hills Ferry Road) was located downstream from the west-side tributaries, but upstream from east-side tributaries that do not receive tile drainwater; and the third site (at Durham Ferry State Recreation Area) was located downstream from both the west-side and east-side tributaries.

Originally, samples from all eight sites were scheduled for analysis of six trace elements--arsenic, boron, chromium, mercury, molybdenum, and selenium. However, due to reductions in funding, we were able to analyze samples from only seven sites (samples from the Tuolumne River were omitted) for three elements--boron, molybdenum, and selenium.

Sample Collection and Storage: Water samples were collected weekly from each site during the two sampling periods. Each sample was collected within 15 cm of the surface as a composite of three grab samples (one each from the left and right banks, and in mid-channel), then duplicate 500-mL samples were filtered under pressure through 0.40 µm polycarbonate membranes. Duplicate 500-mL unfiltered water samples were also collected. The filtered and unfiltered samples were placed in acid-rinsed polyethylene bottles, then acidified with ultrapure nitric acid to pH <2.0 and stored frozen (-10°C).

We attempted to collect triplicate samples of each of the remaining matrices from each site during the two sampling periods. Sediment samples were collected with a standard Peterson dredge. Detrital samples were collected by straining the sediments through 3.2-mm mesh nets, then hand-sorting the remaining materials for particulate organic detritus. Filamentous algae, net plankton, aquatic macroinvertebrates, and fish were sampled with standard gear (e.g., plankton nets, fine-mesh dip nets, gill nets, seines, and electrofishing gear); the algae, macroinvertebrates, and fish were hand-sorted from debris immediately after capture. For biological matrices (excluding fish and crayfish), we attempted to collect composite samples weighing at least 2.0 g and preferably 40 g. For fish and crayfish, we attempted to collect composite samples of 3-5 individuals that collectively weighed at least 40 g. Individual fish were also measured for total length. All samples were wrapped and double-bagged in polyethylene, and stored frozen at -10°C.

Elemental Analyses and Quality Assurance: A total of 688 samples were analyzed for trace elements by the National Fisheries Contaminant Research Center, in Columbia, MO. Except for water, all samples were lyophilized in order to determine the moisture content. For large organisms such as fish, frozen samples were cut into cubes with a Hobart® food-service band saw and ground with a meat grinder. The ground material was thoroughly mixed by making three passes of the entire sample through the meat grinder, then a portion of the homogenized tissue was lyophilized. Some fish samples were too small for frozen homogenization; these samples were cut open with bone shears and lyophilized directly.

After lyophilization was completed, algae, detritus, crayfish, and large (>50 g) fish were dry-blended with an Osterizer® blender. Other matrices (i.e., sediment, plankton, amphipods, and chironomids) did not need any further homogenization following the lyophilization procedure. All of the lyophilized samples were transferred into acid-cleaned glass vials and stored in a desiccator while awaiting further preparation and analysis.

For analysis of boron, water samples were digested with a Teflon®-vessel microwave procedure that employed nitric acid (Saiki and May 1988). Other matrices were digested with a similar procedure, except that hydrogen peroxide was added to the nitric acid. Boron was determined from the digestates by inductively coupled argon plasma emission spectrometry (ICP).

For analysis of molybdenum, all sample matrices were digested according to the procedure used to determine boron. However, after digestion was completed, an aliquot of digestate was preconcentrated by coprecipitation. Detritus was exceptional because molybdenum concentrations were so high that preconcentration of the digestate was not necessary. Molybdenum was determined from the digestates by ICP.

Water samples were prepared for analysis of selenium by digesting with a persulfate oxidation procedure (U.S. Environmental Protection Agency 1983). The remaining matrices were digested with a nitric acid-magnesium nitrate dry-ash procedure (Saiki and May 1988). Selenium was determined by hydride-generation atomic-absorption spectrophotometry with Varian® instrumentation.

Sixty-three groups of samples were processed for boron, molybdenum, and selenium with the following quality controls: procedural blanks, calibration solutions, digestion spikes, analysis spikes, replicate sample preparation and analysis, and appropriate water, tissue, and other quality control materials. In general, there were no unusual problems associated with the chemical determinations. However, several samples of sediment, detritus, and plankton were not successfully analyzed for molybdenum because of quality control failures with the existing methodology. For example, abnormal color and consistency of the sample mixtures were obtained upon coprecipitation of the digestates, presumably from unknown interferences.

Percent recoveries of boron (based on Eastman-Kodak® gelatin, the only material certified for this element) were somewhat variable, extending from 20% on the low side of the range to 6% on the high side of the range. Data for molybdenum from control materials indicated that quantitative recovery was not achieved, with most concentrations being low by 10-25% of the certified values. Some of the losses occurred during the coprecipitation procedure, which is not quantitative (losses of 10% are common) and varies in performance among different matrices. The recoveries of selenium from control materials were generally within the recommended or certified ranges; for those recoveries that exceeded the certified ranges, all except one sample were within 19% of the low end of the range. The exceptional sample measured 52% lower than the certified range.

Analytical precision, as measured by relative standard deviation (RSD), was <25%

for all three elements. Samples exhibiting RSD's of 20-25% contained concentrations of elements between the limit of detection and the limit of quantification, which may explain the relatively poor precision. Most digested spike recoveries ranged from 80% to 120%; however, two recoveries were low (54% and 67%) and one recovery was unusually high (232%). Procedural blanks generally had concentrations that were less than the limit of detection. Analysis spikes, processed to check for matrix enhancement or suppression effects, had recoveries ranging from 82% to 122%. The limits of detection ranged from a low of 0.00031 μg/g for selenium to a high of 11.8 μg/g for boron.

Analysis of Fish Food Habits: Supplemental samples totalling 180 mosquitofish, 261 bluegills, and 100 largemouth bass were collected from all sites in 1986 and 1987 to document the forage items consumed by these fish. Immediately after collection by seining or electrofishing, the fish were preserved in 10% formalin. When time permitted, the preserved fish were dissected, and all materials present from the esophagus to the pyloric sphincter were identified to appropriate taxonomic categories and weighed (damp-dry biomass).

Statistical Analyses: Prior to calculating means or conducting analysis of variance (ANOVA), all percentages (moisture content, frequency of occurrence of food items, etc.) were arcsine-transformed whereas all other values (elemental concentrations, total lengths, weights, etc.) were logarithmically-transformed. When F-values from ANOVA were statistically significant ($P \leq 0.05$), Tukey-Kramer "honestly significant difference" (hsd) tests for samples of unequal sizes were used to compare the means for statistical differences. If trace element concentrations were below the detection limit, a value of half the detection limit was used to facilitate statistical comparisons.

Principal Findings

Moisture content differed significantly ($\underline{P} \leq 0.05$, based on one-way ANOVA's) among the various matrices and collection sites, and between seasons. In order to standardize the moisture content, all concentrations of elements are hereinafter reported on a dry-weight basis, unless indicated otherwise.

Although consistent patterns were not discerned in the concentrations of boron, molybdenum, and selenium measured in various matrices, the concentrations were significantly different ($\underline{P} \leq 0.05$, based on one-way ANOVA's) between samples from the spring and fall in over 3/4 of the comparisons. Therefore, further evaluations of elemental concentrations were made according to season.

Concentrations of boron, molybdenum, and selenium were generally higher in detritus (which is mostly composed of decaying plant material) than in the other matrices (Tables 1-3), although boron also occurred at high concentrations in algae (Table 1). Overall, geometric means of trace elements in food-chain organisms were significantly higher ($\underline{P} \leq 0.05$) at sites exposed to agricultural drainwater (i.e., Mud (north) and Salt sloughs, and the San Joaquin River above Hills Ferry Road; Figures 2-7) than at sites that received little or no drainwater.

The concentrations of boron and molybdenum increased from water to sediments to algae and detritus, then generally decreased from algae and detritus to invertebrates and fish (Tables 1-2 and Figures 2-5). In the case of molybdenum, concentrations in macroinvertebrates were approximately twice as high as in fish (Figures 4-5). According to Phillips and Russo (1978), boron and molybdenum can bioconcentrate in plants; however, they do not readily accumulate in animals, including fish. Based on data from a limited number of field surveys, Saiki and May (1988) suggested that whole freshwater fish typically contain <4 μg/g boron, whereas concentrations of molybdenum in fish are usually <0.6 μg/g. By comparison, bluegills or mosquitofish from three of the sites we sampled (i.e., Mud (north) and Salt sloughs and the San Joaquin River above Hills Ferry Road) contained elevated concentrations of boron, but none of the sites contained elevated concentrations of molybdenum. The effects of elevated boron concentrations in fish are unknown.

With few exceptions, data from our survey indicated that selenium concentrations generally increased from water to sediment to algae to invertebrates (plankton, amphipods, chironomids, and crayfish) to fish (bluegills, mosquitofish, and largemouth bass; Table 3 and Figures 6-7). Detritus was unusual because, although it consisted mostly of decaying plant material, it often contained selenium concentrations that equaled or exceeded those in fish. Saiki and Lowe (1987) reported a similar pattern for selenium concentrations (including high concentrations in detritus) in the aquatic food chain at Kesterson Reservoir. Although several authors (e.g., Cherry and Guthrie 1977; Lemly 1985; Lemly and Smith 1987) have reported that selenium can biomagnify through the aquatic food chain, Kay (1984) argued that conclusive evidence of biomagnification (specifically defined as an increase in the concentration of selenium from one trophic level to the next higher one) is not yet available in the literature.

Lemly and Smith (1987) reported that selenium concentrations >2-5 μg/L in water, ≥4 μg/g in sediment, ≥5 μg/g in food, and ≥12 μg/g in the whole-body of fish can cause reproductive failure and other toxic problems among sensitive species such as centrarchids. Judging from these general guidelines, potentially toxic concentrations of selenium occurred at three sites during our survey: Mud (north) and Salt sloughs, and the San Joaquin River above Hills Ferry Road (Table 3). Selenium concentrations in detritus (up to 23 μg/g) and mosquitofish (up to 17 μg/g) from Mud (north) Slough were especially elevated.

Stomach Contents of Fish: Based on biomass, mosquitofish consumed mostly mollusks, adult dipterans, and adult hymenopterans, which collectively accounted for nearly 100% of the "Other" category in Figure 8. Although unimportant from the standpoint of biomass, many mosquitofish also ate plants (primarily diatoms), microcrustacea (cladocerans, copepods, and ostracods), and chironomid larvae. Bluegills foraged mostly on amphipods, microcrustacea, and chironomid larvae (Figure 8). The primary foods of largemouth bass were fish (mostly unidentified fish remains) and crayfish (Figure 8). In general, these data verify that the aquatic organisms collected for analysis of trace elements were indeed important components of the food chain in the San Joaquin River system.

Conclusions and Research Needs

Our survey indicated that boron, molybdenum, and selenium in water were readily bioconcentrated by filamentous algae. High concentrations of all three elements also occurred in the decaying plant material that composed our samples of detritus. The concentrations of boron and molybdenum decreased from net plankton to macroinvertebrates to fish, suggesting that they do not accumulate in higher trophic levels. In contrast, selenium concentrations generally increased from algae to net plankton to macroinvertebrates to fish in a pattern suggestive of biomagnification. Selenium concentrations in detritus were not expected to exceed the concentrations occurring in algae; this discrepancy cannot be explained from the available data.

As judged by data from other studies (e.g., Lemly and Smith 1987; Saiki and May 1988), the concentrations of boron and selenium were elevated in aquatic food chains from three sites: Salt and Mud (north) sloughs and the San Joaquin River above Hills Ferry Road. Selenium concentrations in water and biota from these sites also exceeded toxic concentrations for selenium-sensitive species such as bluegills, and largemouth bass (Lemly and Smith 1987).

Further research is needed to better understand the environmental consequences of discharging agricultural drainwater into the San Joaquin River and its tributaries. Such studies should include the following:

1. The factors controlling the uptake and excretion of boron and molybdenum in aquatic biota should be better understood. Although these elements apparently do not biomagnify through the food chain, if sufficiently high concentrations can accumulate in aquatic plants and detritus, animals that forage on these lower trophic levels might still be exposed to potentially toxic concentrations. Presently, the toxic thresholds for these elements to fish are unknown.

2. The interactions and possible synergistic effects of elevated levels of boron, molybdenum, and selenium on aquatic biota need to be investigated. Aquatic biota inhabiting drainwater-influenced ecosystems are generally exposed to a mixture of trace elements, of which more than one may occur at elevated concentrations. However, because past studies have typically investigated only one trace element at a time, the effects of these combinations are still poorly understood.

3. Controlled laboratory studies are needed to verify that selenium can biomagnify through aquatic food chains. Also, the mechanisms that allow unusually high concentrations of selenium to accumulate in particulate organic detritus must be better understood, especially because detritus is a significant link in many aquatic food chains.

4. Based on general guidelines for toxic thresholds of selenium in water, sediment, food, and fish tissues, the concentrations of this element are sufficiently elevated to impair the reproduction of centrarchids in at least three localities in the San Joaquin River system.

However, evidence of adverse effects on fish are not available. Studies that attempt to assess the health and reproductive success of local fish populations are needed in order to better evaluate the environmental damage caused by the disposal of seleniferous agricultural drainwater in the San Joaquin River.

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Table 1. Concentrations of boron in water (mg/L), sediment (µg/g dry weight), and biota (µg/g dry weight) from eight sites in the San Joaquin River system during 1987. See Figure 1 for abbreviations and locations of sites. Season, S = spring, F = fall; N/N = number of samples detected/number of samples analyzed; $\bar{X}$ = geometric mean; (min. - max.) = minimum value - maximum value.

		Algae			Amphipoda			Bluegill			Chironomidae			Crayfish			Detritus		
Site	Season	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)
MR	S	0/3	<6.09	(<6.1-<6.1)	0/3	<2.18	(<2.2-<2.2)	0/3	<1.98	(<1.9-<2.0)	0/3	<1.80	(<1.8-<1.8)	0/3	<2.18	(<2.2-<2.2)	3/3	10.6	(10.-11.)
MR	F	3/3	199.	(94.-320.)	3/3	7.30	(6.5-7.8)	0/3	<1.84	(<1.8-<1.8)	3/3	3.89	(2.5-4.9)	2/3	1.21	(<1.4-1.7)	3/3	13.3	(10.-21.)
MSGCR	S	3/3	282.	(230.-330.)	3/3	22.0	(21.-23.)	3/3	7.85	(2.8-15.)	3/3	26.8	(25.-28.)	3/3	22.6	(16.-28.)	3/3	149.	(130.-160.)
MSGCR	F	3/3	204.	(190.-220.)	3/3	19.2	(15.-31.)	2/5	1.71	(<2.0-4.7)	3/3	18.5	(14.-26.)	3/3	9.61	(8.4-11.)	3/3	191.	(180.-200.)
SJRDF	S	3/3	14.2	(8.9-27.)	0/3	<2.18	(<2.2-<2.2)	0/3	<1.99	(<2.0-<2.0)	3/3	6.34	(4.9-8.0)	3/3	5.57	(4.7-6.4)	3/3	50.0	(48.-52.)
SJRDF	F	3/3	42.8	(3.4-180.)	3/3	7.43	(5.3-11.)	0/3	<2.00	(<2.0-<2.0)	3/3	8.12	(7.3-8.7)	3/3	2.43	(2.3-2.6)	3/3	23.7	(20.-29.)
SJRHF	S	3/3	79.1	(54.-110.)	3/3	22.5	(20.-27.)	1/3	1.44	(<1.9-3.2)	3/3	15.9	(11.-20.)	3/3	15.1	(13.-17.)	3/3	171.	(160.-180.)
SJRHF	F	3/3	58.5	(45.-80.)	3/3	15.2	(12.-17.)	0/3	<1.84	(<1.8-<1.8)	3/3	15.4	(13.-18.)	3/3	10.6	(9.7-11.)	3/3	77.4	(71.-83.)
SJRLA	S	2/3	6.75	(<6.1-13.)	0/2	<2.18	(<2.2-<2.2)	0/3	<1.94	(<1.9-<1.9)	3/3	2.37	(1.8-4.4)	3/3	3.82	(3.2-4.6)	3/3	46.5	(39.-54.)
SJRLA	F	3/3	91.7	(82.-110.)	3/3	7.26	(6.9-7.6)	0/4	<1.84	(<1.8-<1.8)	3/3	10.2	(9.5-11.)	3/3	1.95	(1.6-2.9)	3/3	19.4	(16.-23.)
SSSLNWR	S	3/3	43.7	(29.-82.)	3/3	15.8	(15.-16.)	0/3	<1.94	(<1.9-<1.9)	3/3	14.4	(14.-16.)	3/3	13.0	(9.5-16.)	3/3	141.	(140.-150.)
SSSLNWR	F	3/3	50.2	(37.-68.)	3/3	13.8	(11.-17.)	1/4	1.19	(<1.8-2.6)	3/3	19.9	(19.-22.)	3/3	6.74	(5.5-9.3)	3/3	102.	(91.-120.)
SR	S	0/3	<6.09	(<6.1-<6.1)	3/3	3.46	(3.1-4.1)	0/3	<1.99	(<2.0-<2.0)	3/3	2.63	(2.3-3.1)	3/3	3.13	(3.0-3.3)	3/3	22.9	(13.-59.)
SR	F	3/3	3.48	(2.8-4.5)	3/3	7.80	(5.8-10.)	0/3	<2.00	(<2.0-<2.0)	3/3	3.92	(2.5-5.8)	2/2	2.72	(2.0-3.8)	3/3	10.2	(9.6-11.)

Table 1.--Continued.

		Largemouth bass			Mosquitofish			Net plankton			Sediment			Water, filtered			Water, unfiltered		
Site	Season	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)
MR	S	0/3	<1.94	(<1.9-<1.9)	0/3	<1.94	(<1.9-<1.9)	1/3	1.07	(<1.6-1.8)	0/3	<1.79	(<1.8-<1.8)	0/6	<0.090	(<0.09-<0.09)	0/6	<0.090	(<0.09-<0.09)
MR	F	0/4	<1.84	(<1.8-<1.8)	0/3	<1.84	(<1.8-<1.8)	--	--	---	0/3	<3.22	(<3.2-<3.2)	0/6	<0.170	(<0.17-<0.17)	0/6	<0.170	(<0.17-<0.17)
MSGCR	S	2/3	1.97	(<2.0-2.9)	3/3	8.38	(7.8-9.1)	3/3	45.6	(44.-48.)	3/3	6.07	(5.1-7.8)	6/6	2.92	(1.7-3.6)	6/6	2.71	(1.7-3.3)
MSGCR	F	0/3	<2.00	(<2.0-<2.0)	3/3	7.78	(6.4-9.8)	3/3	25.2	(23.-29.)	3/3	11.6	(6.7-16.)	6/6	1.46	(0.83-3.4)	6/6	1.58	(0.80-3.6)
SJRDF	S	0/3	<1.99	(<2.0-<2.0)	0/3	<1.99	(<2.0-<2.0)	3/3	8.69	(7.5-10.)	0/3	<1.79	(<1.8-<1.8)	5/6	0.395	(<0.26-0.57)	6/6	0.381	(<0.26-0.60)
SJRDF	F	0/3	<2.00	(<2.0-<2.0)	3/3	3.16	(3.0-3.5)	3/3	16.5	(15.-19.)	0/3	<3.22	(<3.2-<3.2)	6/6	0.412	(0.33-0.55)	6/6	0.402	(0.33-0.43)
SJRHF	S	0/3	<1.94	(<1.9-<1.9)	3/3	5.79	(5.2-6.6)	3/3	21.5	(11.-31.)	3/3	2.90	(2.2-4.3)	6/6	1.96	(1.4-2.5)	6/6	1.83	(1.3-2.8)
SJRHF	F	0/3	<1.84	(<1.8-<1.8)	3/3	4.26	(3.5-5.9)	3/3	39.8	(31.-49.)	0/3	<3.22	(<3.2-<3.2)	6/6	1.16	(0.91-2.2)	6/6	1.24	(0.84-2.2)
SJRLA	S	0/3	<1.94	(<1.9-<1.9)	1/3	1.28	(<2.0-2.1)	3/3	6.57	(5.9-8.0)	1/3	1.40	(<1.8-3.4)	4/6	0.102	(<0.09-0.25)	5/6	0.109	(<0.09-0.23)
SJRLA	F	0/3	<1.88	(<1.8-<2.0)	0/3	<1.84	(<1.8-<1.8)	--	--	---	2/3	3.26	(<3.2-6.5)	2/6	0.118	(<0.17-0.26)	1/6	0.098	(<0.17-0.20)
SSSLNWR	S	0/3	<1.96	(<1.9-<2.0)	3/3	3.05	(2.2-4.2)	3/3	8.24	(7.4-8.8)	3/3	6.92	(4.9-9.2)	6/6	1.89	(1.4-3.5)	6/6	1.83	(1.4-2.7)
SSSLNWR	F	0/4	<1.84	(<1.8-<1.8)	3/3	4.78	(4.0-6.4)	--	--	---	2/3	2.73	(<3.2-3.8)	6/6	0.943	(0.47-1.9)	6/6	0.995	(0.58-1.5)
SR	S	0/3	<1.99	(<2.0-<2.0)	0/3	<1.99	(<2.0-<2.0)	0/3	<1.63	(<1.6-<1.6)	0/2	<1.79	(<1.8-<1.8)	0/6	<0.260	(<0.26-<0.26)	0/6	<0.260	(<0.26-<0.26)
SR	F	0/3	<2.00	(<2.0-<2.0)	0/3	<2.00	(<2.0-<2.0)	--	--	---	0/3	<3.22	(<3.2-<3.2)	0/6	<0.120	(<0.12-<0.12)	0/6	<0.120	(<0.12-<0.12)

Table 2. Concentrations of molybdenum in water (mg/L), sediment (µg/g dry weight), and biota (µg/g dry weight) from eight sites in the San Joaquin River system during 1987. See Figure 1 for abbreviations and locations of sites. Season, S = spring, F = fall; N/N = number of samples detected/number of samples analyzed; $\bar{X}$ = geometric mean; (min. - max.) = minimum value - maximum value.

		Algae			Amphipoda			Bluegill			Chironomidae			Crayfish			Detritus		
Site	Season	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)
MR	S	3/3	0.900	(0.86-0.92)	1/3	0.428	(<0.65-0.74)	3/3	0.351	(0.30-0.40)	3/3	0.824	(0.62-1.2)	0/3	<0.500	(<0.50-<0.50)	--	--	---
MR	F	2/3	1.40	(<0.30-4.7)	2/3	0.417	(<0.50-0.57)	0/3	<0.490	(<0.49-<0.49)	1/3	0.248	(<0.38-0.42)	2/3	0.402	(<0.50-0.51)	3/3	5.12	(4.6-6.0)
MSGCR	S	3/3	1.59	(1.1-1.9)	0/3	<0.650	(<0.65-<0.65)	0/3	<0.890	(<0.89-<0.89)	3/3	1.33	(0.73-3.5)	1/3	0.316	(<0.50-0.51)	--	--	---
MSGCR	F	3/3	0.916	(0.36-1.6)	3/3	0.562	(0.51-0.59)	2/5	0.141	(<0.21-0.22)	2/3	0.535	(<0.38-0.96)	2/3	0.435	(<0.50-0.66)	3/3	19.4	(18.-20.)
SJROF	S	3/3	0.910	(0.74-1.0)	0/3	<0.650	(<0.65-<0.65)	0/3	<0.890	(<0.89-<0.89)	0/3	<0.420	(<0.42-<0.42)	0/3	<0.500	(<0.50-<0.50)	--	--	---
SJROF	F	3/3	0.296	(<0.30-0.54)	2/3	0.484	(<0.50-0.77)	2/3	0.186	(<0.21-0.28)	0/3	<0.380	(<0.38-<0.38)	1/3	0.317	(<0.50-0.51)	3/3	2.42	(2.2-2.7)
SJRHF	S	2/3	0.569	(<0.39-1.1)	0/3	<0.650	(<0.65-<0.65)	0/3	<0.220	(<0.22-<0.22)	1/3	0.328	(<0.42-0.80)	1/3	0.332	(<0.50-0.58)	--	--	---
SJRHF	F	0/3	<0.300	(<0.30-<0.30)	3/3	0.567	(0.51-0.70)	0/3	<0.453	(<0.38-<0.49)	0/3	<0.380	(<0.38-<0.38)	0/3	<0.500	(<0.50-<0.50)	3/3	9.59	(8.9-11.)
SJRLA	S	--	--	---	1/2	1.19	(<0.65-4.4)	2/3	0.212	(<0.22-0.36)	1/3	0.269	(<0.42-0.44)	2/3	0.460	(<0.50-0.67)	--	--	---
SJRLA	F	3/3	<0.300	(<0.30-<0.30)	3/3	0.640	(0.56-0.69)	0/4	<0.490	(<0.49-<0.49)	1/3	0.269	(<0.38-0.54)	1/3	0.315	(<0.50-0.50)	3/3	30.7	(17.-43.)
SSSLNWR	S	3/3	0.837	(0.62-1.1)	3/3	0.884	(0.66-1.1)	3/3	0.417	(0.36-0.48)	0/3	<0.420	(<0.42-<0.42)	2/3	0.834	(<0.50-2.2)	--	--	---
SSSLNWR	F	3/3	0.785	(0.60-0.96)	2/3	0.537	(<0.50-1.1)	3/4	0.511	(<0.49-0.78)	0/3	<0.380	(<0.38-<0.38)	0/3	<0.500	(<0.50-<0.50)	3/3	3.96	(3.6-4.4)
SR	S	3/3	2.04	(1.8-2.5)	0/3	<0.650	(<0.65-<0.65)	0/3	<0.890	(<0.89-<0.89)	0/3	<0.420	(<0.42-<0.42)	0/3	<0.500	(<0.50-<0.50)	--	--	---
SR	F	3/3	0.457	(0.42-0.54)	1/3	0.367	(<0.50-0.79)	3/3	0.284	(0.24-0.34)	1/3	0.259	(<0.38-0.48)	0/2	<0.500	(<0.50-<0.50)	3/3	2.72	(2.7-2.8)

Table 2.--Continued.

		Largemouth bass			Mosquitofish			Net plankton			Sediment			Water, filtered			Water, unfiltered		
Site	Season	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)
MR	S	1/3	0.143	(<0.22-0.24)	0/3	<0.220	(<0.22-<0.22)	1/3	0.374	(<0.46-0.99)	1/3	0.335	(<0.52-0.56)	0/6	<0.010	(<0.01-<0.01)	0/6	<0.010	(<0.01-<0.01)
MR	F	0/4	<0.490	(<0.49-<0.49)	0/3	<0.490	(<0.49-<0.49)	--	--	---	--	--	---	0/6	<0.008	(<0.008-<0.008)	1/6	0.005	(<0.008-0.009)
MSGCR	S	0/3	<0.900	(<0.89-<0.90)	0/3	<0.890	(<0.89-<0.89)	3/3	0.978	(0.69-1.3)	3/3	0.599	(0.56-0.62)	1/6	0.006	(<0.01-0.02)	1/6	0.006	(<0.01-0.02)
MSGCR	F	0/3	<0.210	(<0.21-<0.21)	0/3	<0.210	(<0.21-<0.21)	--	--	---	--	--	---	3/6	0.013	(<0.008-0.04)	4/6	0.014	(<0.008-0.04)
SJROF	S	0/3	<0.890	(<0.89-<0.89)	0/3	<0.890	(<0.89-<0.89)	0/3	<0.460	(<0.46-<0.46)	0/3	<0.518	(<0.52-<0.52)	1/6	0.004	(<0.007-0.009)	0/6	<0.007	(<0.007-<0.007)
SJROF	F	0/3	<0.210	(<0.21-<0.21)	0/3	<0.210	(<0.21-<0.21)	--	--	---	--	--	---	0/6	<0.021	(<0.02-<0.02)	0/6	<0.021	(<0.02-<0.02)
SJRMF	S	0/3	<0.220	(<0.22-<0.22)	0/2	<0.220	(<0.22-<0.22)	1/3	0.311	(<0.46-0.57)	1/3	0.346	(<0.52-0.62)	0/6	<0.009	(<0.009-<0.009)	0/6	<0.009	(<0.009-<0.009)
SJRMF	F	0/3	<0.490	(<0.49-<0.49)	0/3	<0.490	(<0.49-<0.49)	--	--	---	--	--	---	0/6	<0.016	(<0.02-<0.02)	0/6	<0.016	(<0.02-<0.02)
SJRLA	S	0/3	<0.220	(<0.22-<0.22)	0/3	<0.900	(<0.90-<0.90)	0/3	<0.460	(<0.46-<0.46)	1/3	0.419	(<0.52-1.1)	0/6	<0.010	(<0.009-<0.01)	0/6	<0.010	(<0.009-<0.01)
SJRLA	F	0/3	<0.467	(<0.42-<0.49)	0/3	<0.490	(<0.49-<0.49)	--	--	---	--	--	---	1/6	0.005	(<0.008-0.02)	2/6	0.006	(<0.008-0.01)
SSSLNWR	S	0/3	<0.217	(<0.21-<0.22)	0/3	<0.220	(<0.22-<0.22)	1/3	0.290	(<0.46-0.46)	3/3	0.904	(0.74-1.2)	0/6	<0.010	(<0.007-<0.01)	0/6	<0.010	(<0.007-<0.01)
SSSLNWR	F	0/4	<0.490	(<0.49-<0.49)	0/3	<0.490	(<0.49-<0.49)	--	--	---	--	--	---	0/6	<0.016	(<0.02-<0.02)	0/6	<0.016	(<0.02-<0.02)
SR	S	0/3	<0.890	(<0.89-<0.89)	0/3	<0.890	(<0.89-<0.89)	0/3	<0.460	(<0.46-<0.46)	0/2	<0.518	(<0.52-<0.52)	0/6	<0.007	(<0.007-<0.007)	0/6	<0.007	(<0.007-<0.007)
SR	F	0/3	<0.210	(<0.21-<0.21)	0/3	<0.210	(<0.21-<0.21)	--	--	---	--	--	---	0/6	<0.021	(<0.02-<0.02)	0/6	<0.021	(<0.02-<0.02)

Table 3. Concentrations of selenium in water (mg/L), sediment (µg/g dry weight), and biota (µg/g dry weight) from eight sites in the San Joaquin River system during 1987. See Figure 1 for abbreviations and locations of sites. Season, S = spring, F = fall; N/N = number of samples detected/number of samples analyzed; $\bar{X}$ = geometric mean; (min. - max.) = minimum value - maximum value.

		Algae			Amphipoda			Bluegill			Chironomidae			Crayfish			Detritus		
Site	Season	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)
MR	S	3/3	0.151	(0.15-0.16)	3/3	0.882	(0.76-0.95)	3/3	1.42	(1.3-1.6)	3/3	1.02	(0.93-1.1)	3/3	0.834	(0.67-0.95)	3/3	1.08	(1.1-1.1)
MR	F	3/3	0.187	(0.09-0.29)	3/3	0.444	(0.40-0.47)	3/3	2.16	(1.7-2.5)	3/3	0.582	(0.57-0.59)	3/3	0.667	(0.53-0.77)	3/3	1.16	(0.91-1.4)
MSGCR	S	3/3	1.63	(1.2-2.0)	3/3	3.25	(3.2-3.3)	5/5	4.96	(4.4-5.5)	3/3	7.17	(6.4-7.9)	3/3	4.39	(3.9-5.2)	3/3	7.85	(7.3-8.3)
MSGCR	F	3/3	7.41	(7.2-7.5)	3/3	4.55	(3.8-6.2)	3/3	6.39	(4.2-8.3)	3/3	8.89	(6.8-14.)	3/3	5.19	(4.2-6.0)	3/3	22.2	(21.-23.)
SJRDF	S	3/3	0.500	(0.35-0.89)	3/3	1.06	(1.0-1.1)	3/3	1.89	(1.8-2.0)	3/3	1.58	(1.6-1.6)	3/3	1.32	(1.1-1.6)	3/3	1.28	(1.3-1.3)
SJRDF	F	3/3	0.387	(0.15-0.68)	3/3	1.52	(1.1-2.6)	3/3	1.99	(1.8-2.1)	3/3	1.46	(1.3-1.7)	3/3	0.766	(0.63-1.1)	3/3	1.23	(1.1-1.5)
SJRHF	S	3/3	1.33	(0.87-1.7)	3/3	2.83	(2.7-2.9)	3/3	2.69	(2.5-2.9)	3/3	4.06	(2.4-5.4)	3/3	1.89	(1.6-2.2)	3/3	3.43	(3.4-3.5)
SJRHF	F	3/3	1.19	(1.1-1.3)	3/3	3.79	(3.5-4.2)	3/3	3.33	(2.8-3.7)	3/3	6.02	(5.1-6.8)	3/3	1.72	(1.4-2.2)	3/3	6.57	(6.4-6.9)
SJRLA	S	3/3	0.100	(0.09-0.10)	3/3	1.29	(1.3-1.3)	3/3	1.38	(1.2-1.7)	3/3	1.01	(0.85-1.4)	3/3	0.737	(0.56-0.86)	3/3	0.580	(0.53-0.65)
SJRLA	F	3/3	0.344	(0.28-0.39)	3/3	0.889	(0.76-0.97)	4/4	0.871	(0.77-0.96)	3/3	0.473	(0.36-0.54)	3/3	0.503	(0.44-0.58)	3/3	0.419	(0.29-0.51)
SSSLNWR	S	3/3	2.43	(1.5-3.4)	3/3	3.72	(3.6-3.8)	3/3	4.32	(3.9-4.7)	3/3	6.91	(5.8-7.8)	3/3	3.17	(2.2-4.0)	3/3	7.87	(7.6-8.1)
SSSLNWR	F	3/3	0.380	(0.27-0.46)	3/3	3.36	(3.1-3.5)	4/4	4.54	(4.2-4.9)	3/3	5.41	(5.0-5.7)	3/3	3.14	(2.5-4.6)	3/3	8.85	(8.7-9.0)
SR	S	3/3	0.242	(0.23-0.26)	3/3	1.08	(1.1-1.1)	3/3	1.22	(1.2-1.3)	3/3	0.84	(0.77-0.92)	3/3	0.848	(0.81-0.88)	3/3	0.713	(0.59-0.80)
SR	F	3/3	0.065	(0.05-0.09)	3/3	1.05	(0.91-1.1)	3/3	1.17	(0.74-2.3)	3/3	0.53	(0.40-0.67)	2/2	0.874	(0.84-0.91)	3/3	0.804	(0.75-0.89)

Table 3.--Continued.

Site	Season	Largemouth bass			Mosquitofish			Net plankton			Sediment			Water, filtered			Water, unfiltered		
		N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)	N/N	$\bar{X}$	(min. - max.)
MR	S	3/3	1.36	(1.3-1.5)	3/3	1.32	(1.3-1.4)	3/3	1.50	(1.2-2.3)	0/3	<0.083	(<0.08-<0.08)	3/6	0.0002	(<0.0003-0.0004)	1/6	0.0002	(<0.0003-0.0004)
MR	F	4/4	0.997	(0.95-1.0)	3/3	1.02	(0.99-1.1)	1/1	1.2	---	2/3	0.090	(<0.07-0.17)	2/6	0.0003	(<0.0005-0.0005)	3/6	0.0004	(<0.0005-0.0006)
MSGCR	S	3/3	6.85	(5.5-8.6)	3/3	10.7	(11.-11.)	3/3	5.36	(5.3-5.5)	3/3	0.196	(0.16-0.24)	6/6	0.009	(0.003-0.02)	6/6	0.009	(0.003-0.02)
MSGCR	F	3/3	6.76	(2.8-11.)	3/3	16.0	(16.-17.)	3/3	2.44	(2.3-2.6)	3/3	1.92	(1.0-3.0)	6/6	0.003	(0.0009-0.02)	5/6	0.002	(<0.001-0.02)
SJROF	S	3/3	1.71	(1.6-1.8)	3/3	1.98	(1.9-2.0)	3/3	1.81	(1.8-1.9)	3/3	0.040	(0.03-0.05)	5/6	0.002	(<0.0008-0.004)	5/6	0.002	(<0.008 0.003)
SJROF	F	3/3	1.77	(1.6-2.1)	3/3	1.74	(1.7-1.8)	3/3	1.60	(1.5-1.7)	1/3	0.046	(<0.07-0.08)	5/6	0.001	(<0.006-0.001)	6/6	0.001	(0.007-0.002)
SJRHF	S	3/3	2.39	(2.3-2.6)	3/3	2.19	(2.1-2.2)	3/3	4.27	(3.8-5.4)	3/3	0.118	(0.10-0.15)	6/6	0.011	(0.007-0.02)	6/6	0.011	(0.007-0.02)
SJRHF	F	3/3	2.23	(1.7-3.0)	3/3	4.53	(4.5-4.6)	3/3	2.63	(2.4-3.0)	1/3	0.045	(<0.07-0.07)	5/6	0.003	(<0.002-0.006)	5/6	0.003	(<0.002-0.006)
SJRLA	S	3/3	1.83	(1.6-2.0)	3/3	1.33	(1.3-1.4)	3/3	1.31	(1.4-1.4)	3/3	0.073	(0.04-0.25)	0/6	<0.0008	(<0.0008-<0.0008)	1/6	0.0005	(<0.0008-0.0009)
SJRLA	F	3/3	0.803	(0.77-0.83)	3/3	0.954	(0.92-0.99)	1/1	1.4	---	2/3	0.135	(<0.07-0.27)	0/6	<0.0006	(<0.0005-<0.0007)	0/6	<0.0006	(<0.0005-<0.0007)
SSSLNWR	S	3/3	4.00	(3.6-4.7)	3/3	4.94	(4.6-5.2)	3/3	4.42	(4.1-4.7)	3/3	0.473	(0.39-0.66)	6/6	0.013	(0.01-0.03)	6/6	0.014	(0.010-0.03)
SSSLNWR	F	4/4	4.72	(3.9-5.3)	3/3	4.47	(4.3-4.7)	3/3	4.53	(4.3-4.8)	3/3	0.344	(0.30-0.37)	5/6	0.003	(<0.0007-0.009)	6/6	0.003	(0.008 0.008)
SR	S	3/3	0.997	(0.92-1.2)	3/3	1.02	(0.98-1.1)	3/3	1.38	(1.4-1.4)	0/3	<0.083	(<0.08-<0.08)	2/6	0.0003	(<0.0003-0.0006)	3/6	0.0004	(<0.0003-0.0007)
SR	F	3/3	0.862	(0.83-0.88)	3/3	0.900	(0.89-0.91)	1/1	0.63	---	2/3	0.060	(<0.07-0.09)	1/6	0.0008	(<0.001-0.007)	0/6	<0.001	(<0.001-<0.001)

Figure 1. Locations of sampling sites. Site names and abbreviations are as follows: (1) MR, Merced River at George J. Hatfield State Recreation Area; (2) SR, Stanislaus River at Caswell Memorial State Park; (3) SSSLNWR, Salt Slough at the San Luis National Wildlife Refuge; (4) MSGCR, Mud Slough at Gun Club Road; (5) SJRLA, San Joaquin River at Highway 165 (Lander Avenue); (6) SJRHF, San Joaquin River above Hills Ferry Road (J-18); and (7) SJRDF, San Joaquin River at Durham Ferry State Recreation Area.

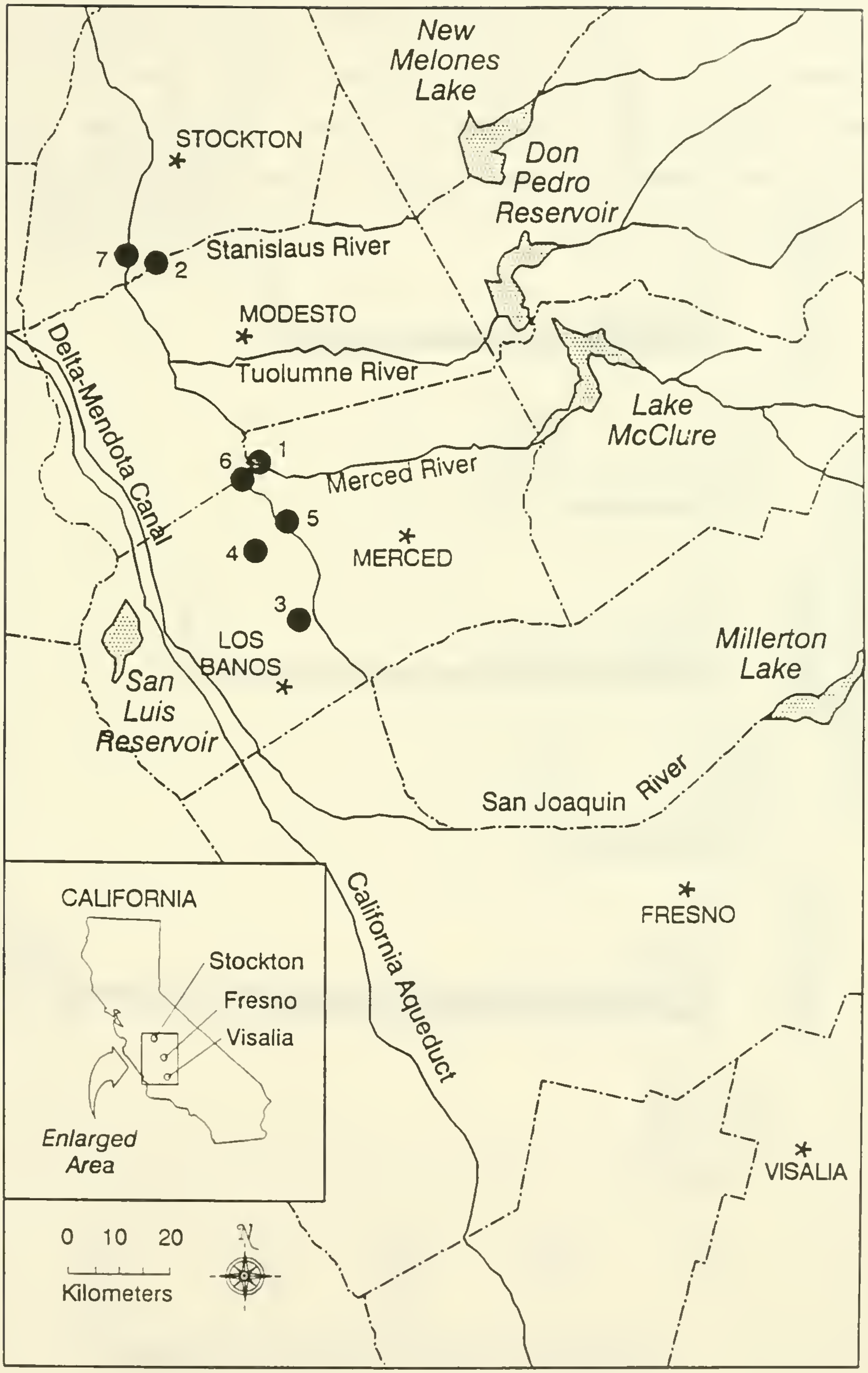
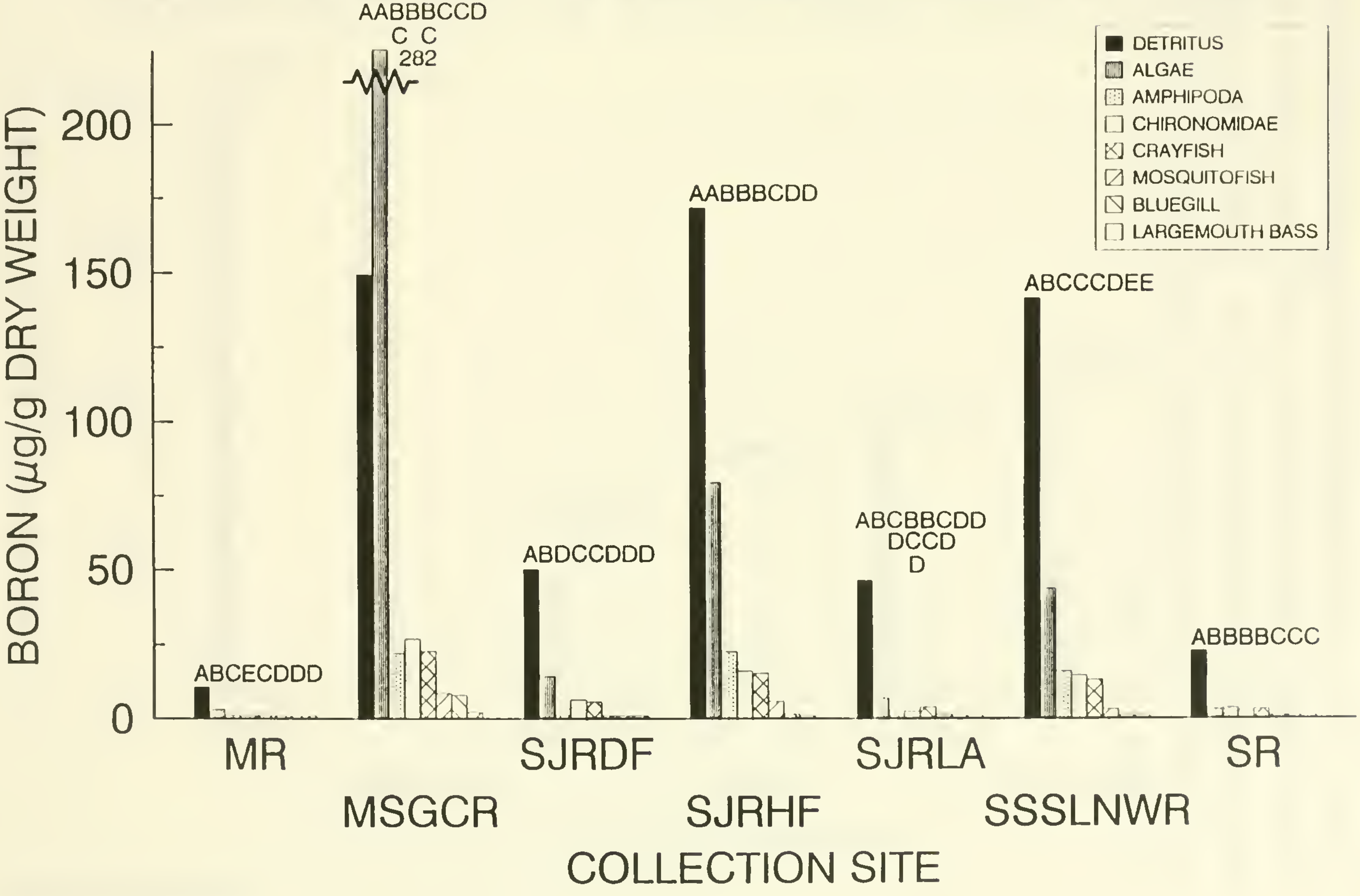

New Melones Lake
Don Pedro Reservoir
STOCKTON
Stanislaus River
7
2
MODESTO
Tuolumne River
Delta-Mendota Canal
Lake McClure
1
6
Merced River
5
MERCED
4
3
LOS BANOS
San Luis Reservoir
Millerton Lake
San Joaquin River
FRESNO
California Aqueduct
CALIFORNIA
Stockton
Fresno
Visalia
Enlarged Area
VISALIA
0 10 20
Kilometers
N

Figure 2. Geometric mean concentrations (μg/g dry weight) of boron in food chain organisms from selected sites in the San Joaquin River system during spring, 1987. See Figure 1 for abbreviations and locations of sites. Within each collection site, bars with the same capitol letter are not significantly different (P >0.05).

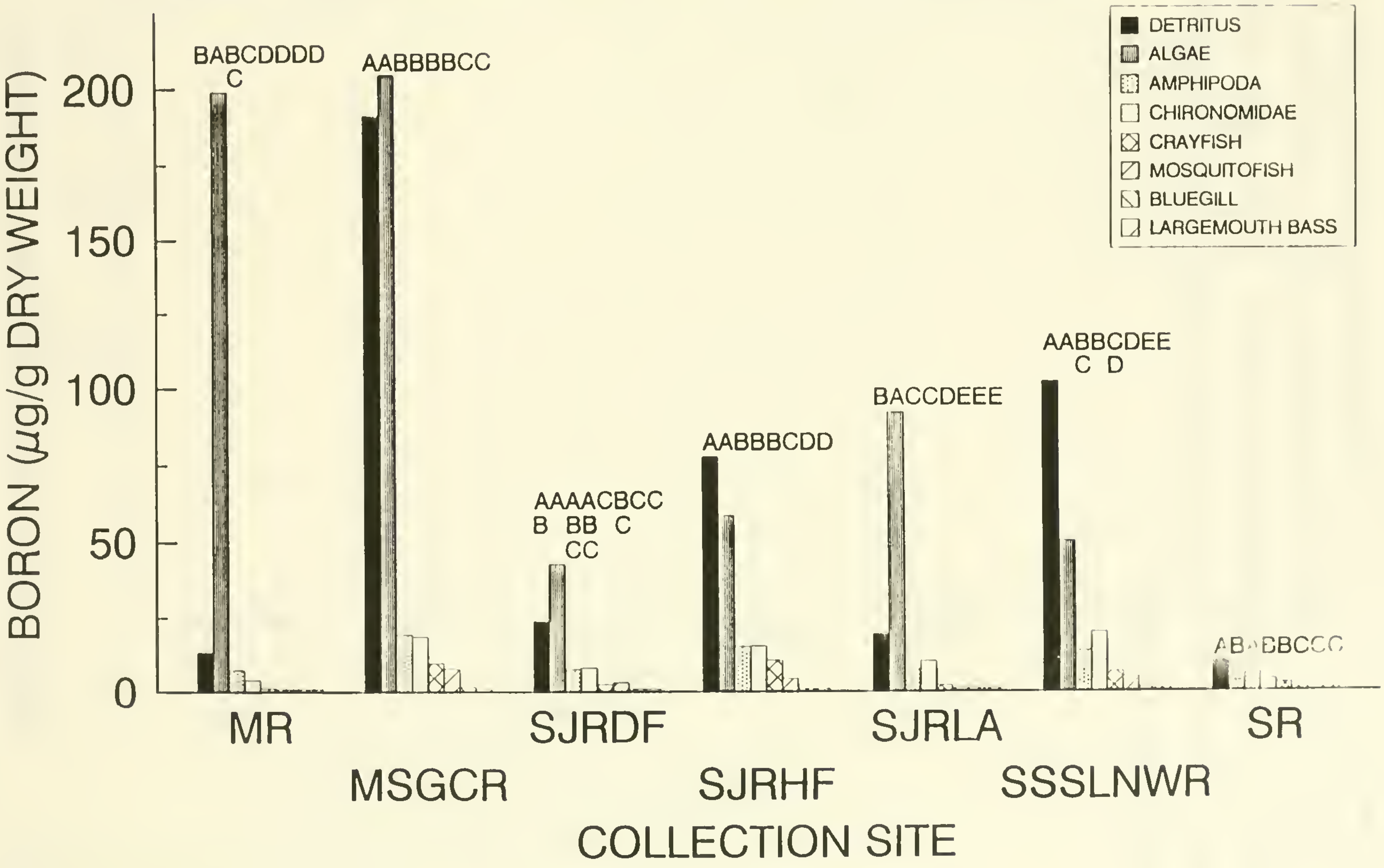
BORON CONCENTRATIONS IN FOOD CHAIN ORGANISMS COLLECTED FROM THE SAN JOAQUIN VALLEY IN 1987



SPRING COLLECTIONS

Figure 3. Geometric mean concentrations (μg/g dry weight) of boron in food chain organisms from selected sites in the San Joaquin River system during fall, 1987. See Figure 1 for abbreviations and locations of sites. Within each collection site, bars with the same capitol letter are not significantly different (P >0.05).

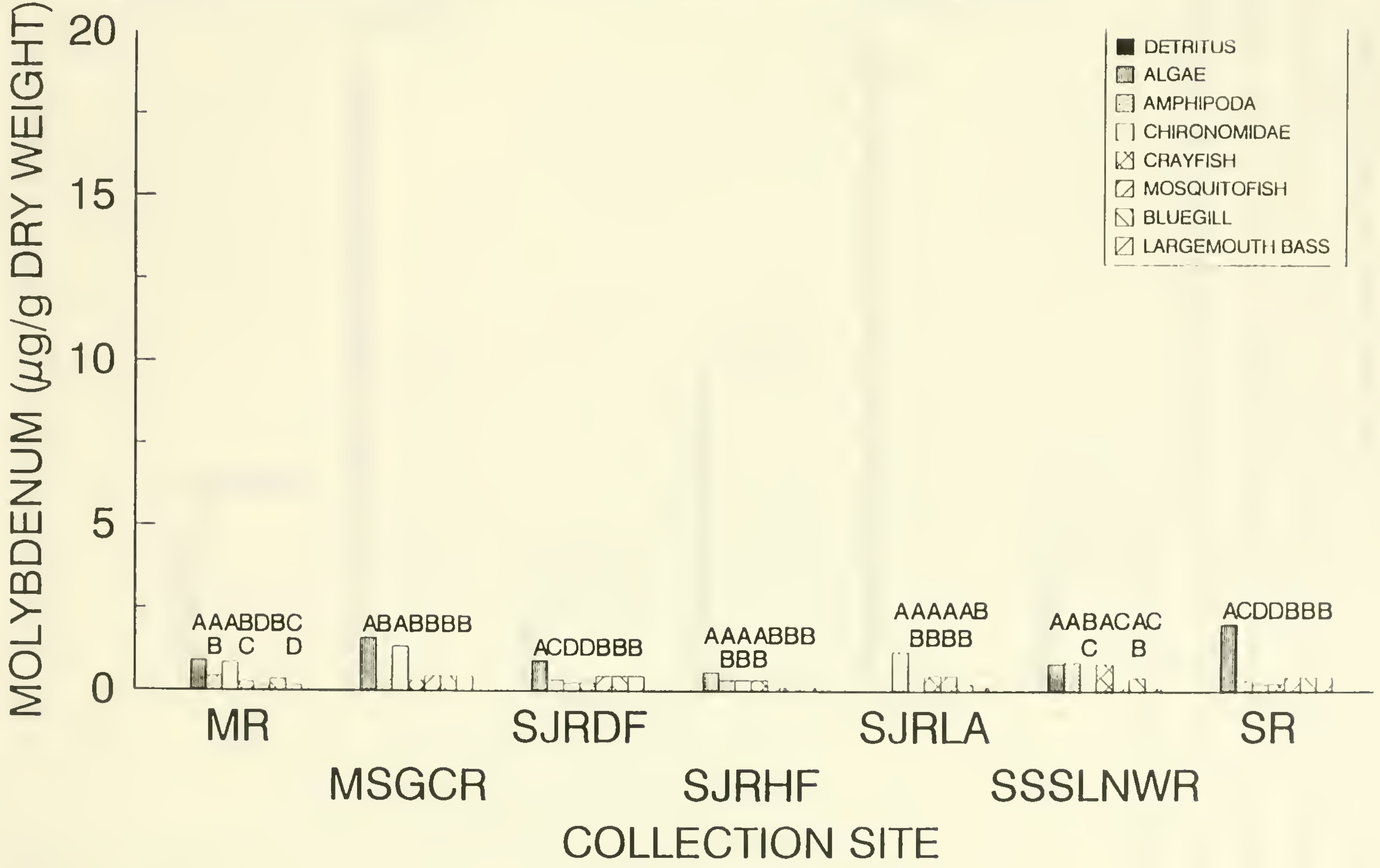
BORON CONCENTRATIONS IN FOOD CHAIN ORGANISMS COLLECTED FROM THE SAN JOAQUIN VALLEY IN 1987



FALL COLLECTIONS

Figure 4. Geometric mean concentrations (μg/g dry weight) of molybdenum in food chain organisms from selected sites in the San Joaquin River system during spring, 1987. See Figure 1 for abbreviations and locations of sites. Within each collection site, bars with the same capitol letter are not significantly different ($\underline{P} > 0.05$).

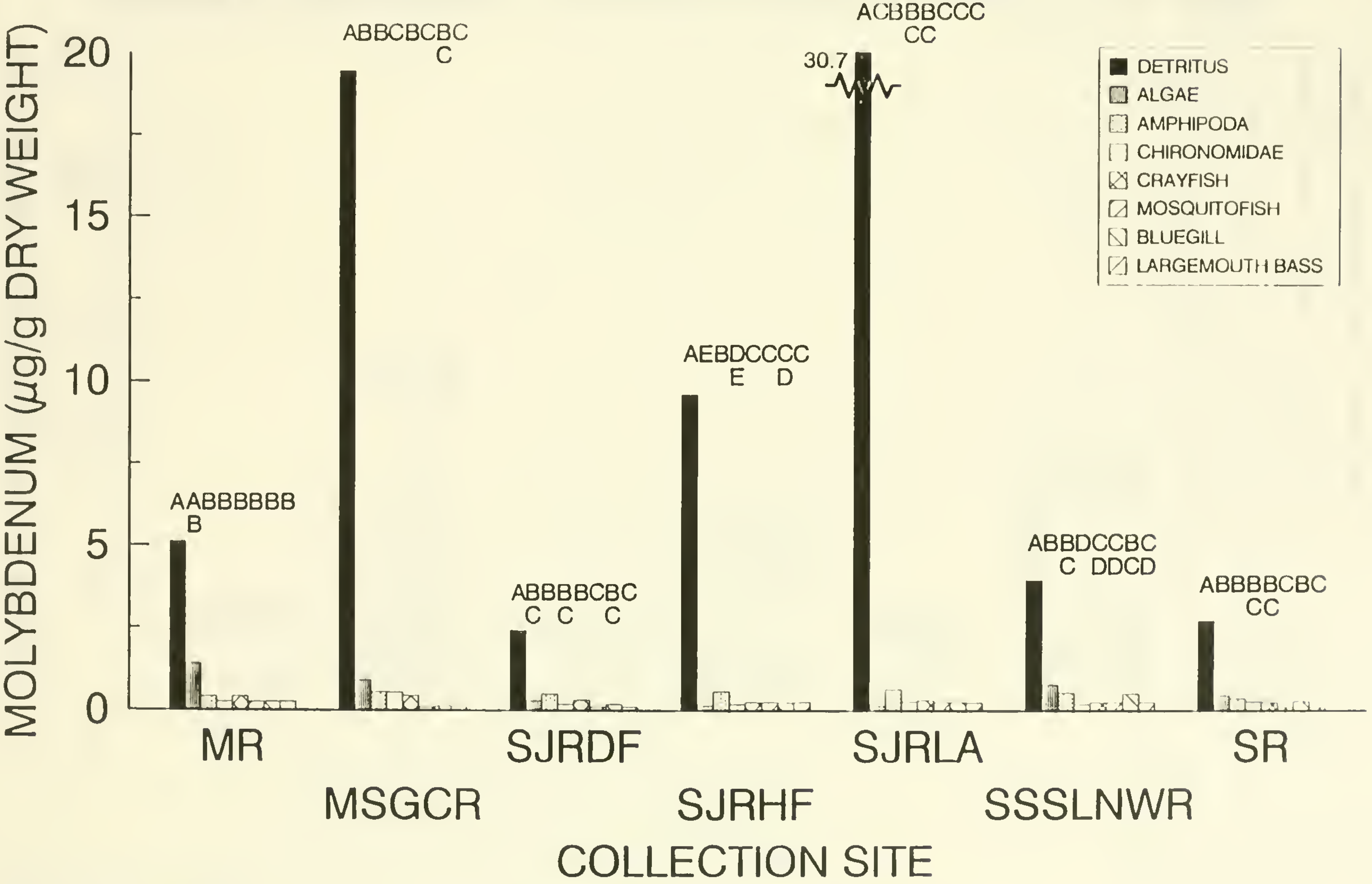
MOLYBDENUM CONCENTRATION IN FOOD CHAIN ORGANISMS COLLECTED FROM THE SAN JOAQUIN VALLEY IN 1987



SPRING COLLECTIONS

Figure 5. Geometric mean concentrations (μg/g dry weight) of molybdenum in food chain organisms from selected sites in the San Joaquin River system during fall, 1987. See Figure 1 for abbreviations and locations of sites. Within each collection site, bars with the same capitol letter are not significantly different (P >0.05).

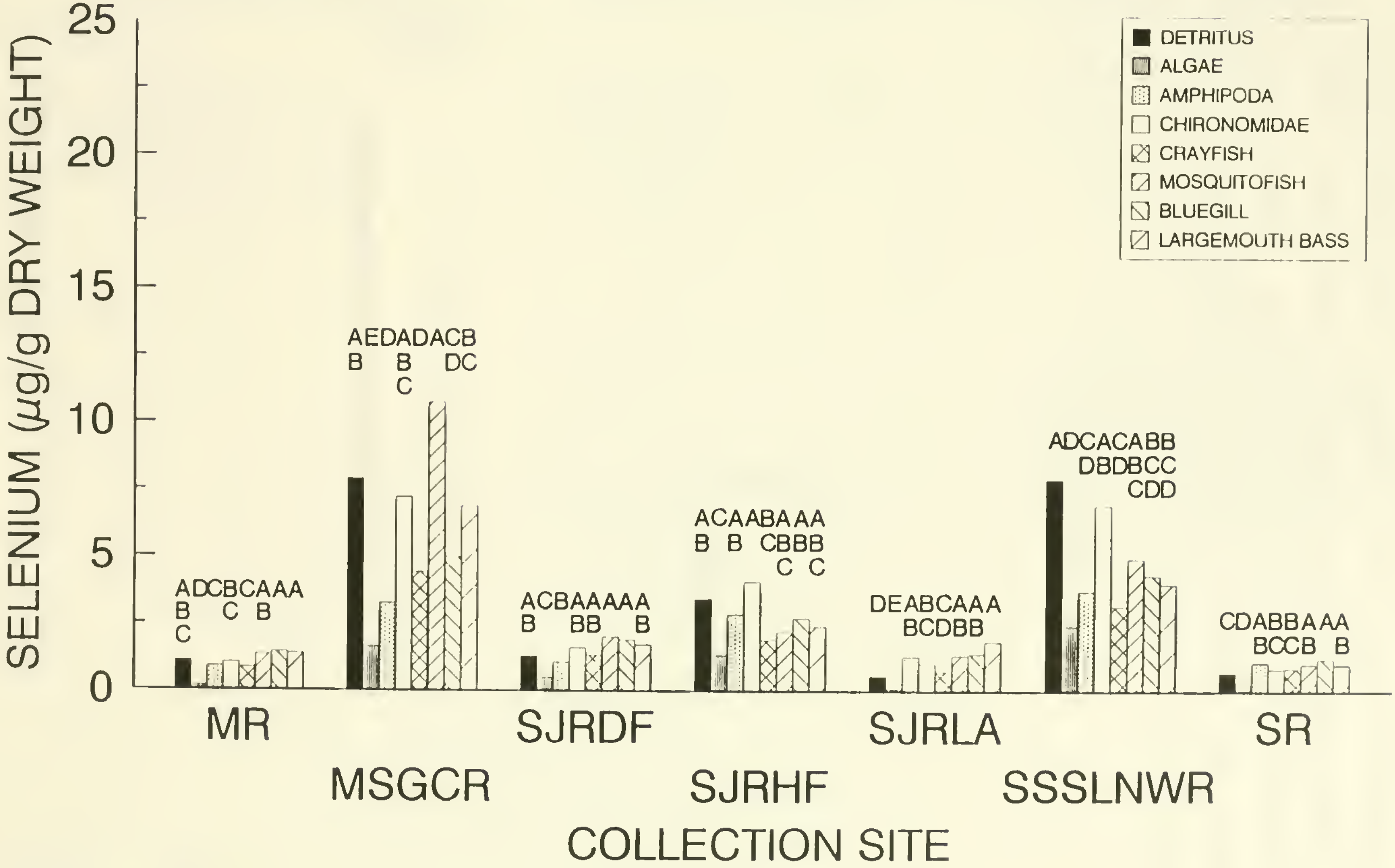
MOLYBDENUM CONCENTRATION IN FOOD CHAIN ORGANISMS COLLECTED FROM THE SAN JOAQUIN VALLEY IN 1987



FALL COLLECTIONS

Figure 6. Geometric mean concentrations (μg/g dry weight) of selenium in food chain organisms from selected sites in the San Joaquin River system during spring, 1987. See Figure 1 for abbreviations and locations of sites. Within each collection site, bars with the same capitol letter are not significantly different ($P > 0.05$).

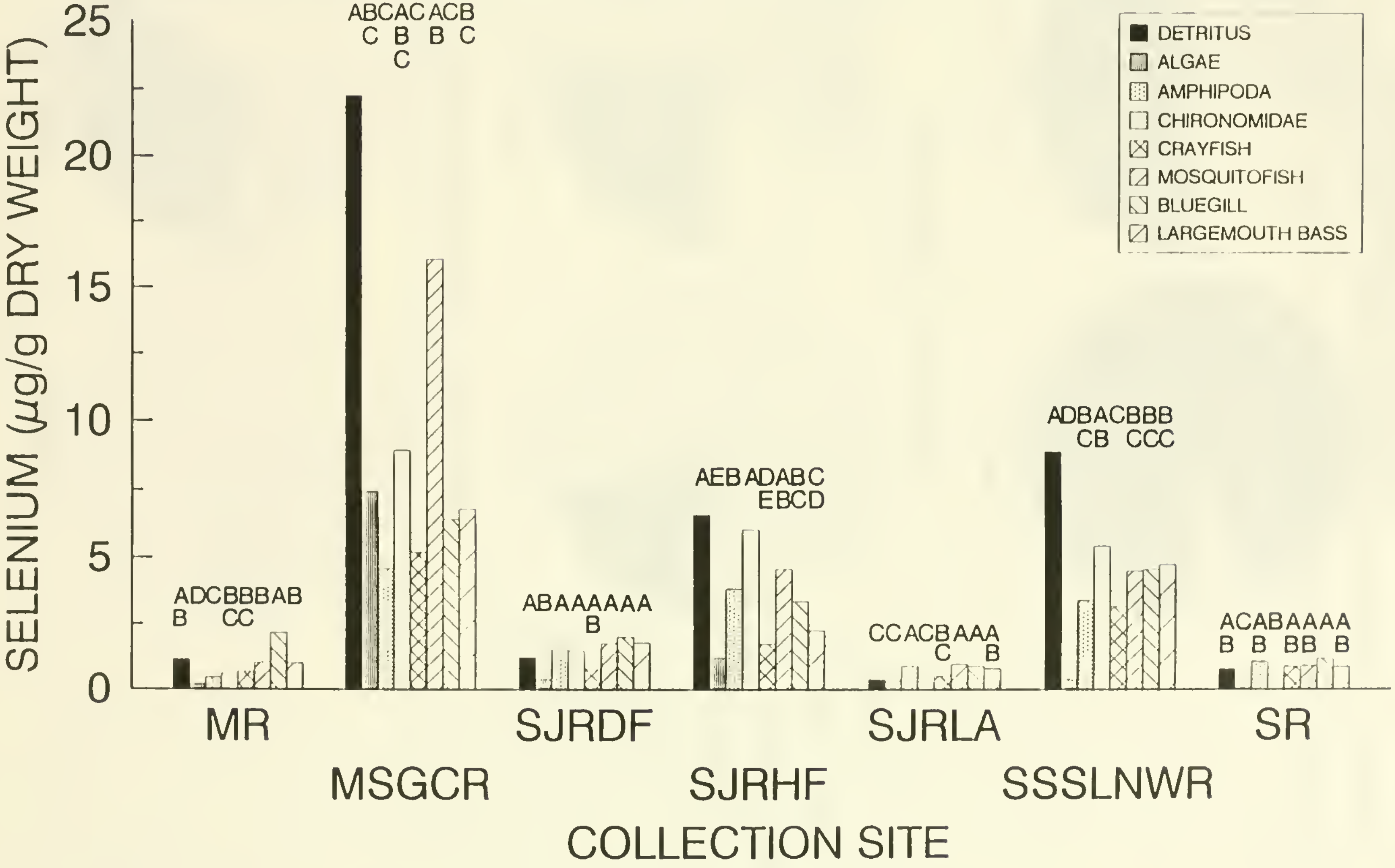
SELENIUM CONCENTATIONS IN FOOD CHAIN ORGANISMS COLLECTED FROM THE SAN JOAQUIN VALLEY IN 1987



SPRING COLLECTIONS

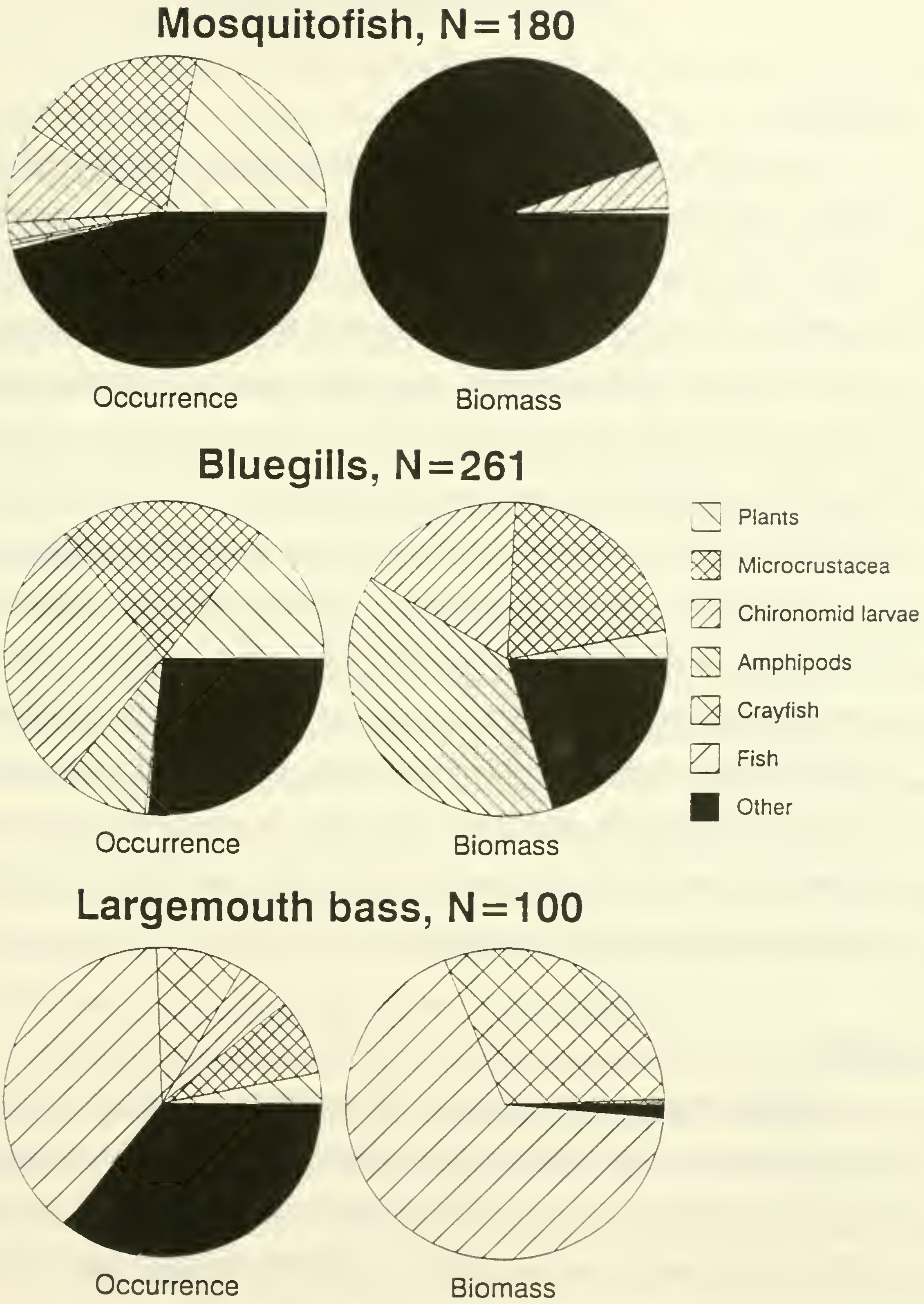
Figure 7. Geometric mean concentrations (μg/g dry weight) of selenium in food chain organisms from selected sites in the San Joaquin River system during fall, 1987. See Figure 1 for abbreviations and locations of sites. Within each collection site, bars with the same capitol letter are not significantly different ($P > 0.05$).

SELENIUM CONCENTATIONS IN FOOD CHAIN ORGANISMS COLLECTED FROM THE SAN JOAQUIN VALLEY IN 1987



FALL COLLECTIONS

Figure 8. Frequency of occurrence and biomass of food items contained in stomachs of mosquitofish, bluegills, and largemouth bass from selected reaches of the San Joaquin River system, 1986-1987.


Mosquitofish, N=180
Occurrence
Biomass
Bluegills, N=261
Occurrence
Biomass
Largemouth bass, N=100
Occurrence
Biomass
Plants
Microcrustacea
Chironomid larvae
Amphipods
Crayfish
Fish
Other

SELENIUM SPECIATION STUDIES

Introduction

As part of the San Joaquin Valley Drainage Program investigations, the National Fisheries Contaminant Research Center conducted research on the bioaccumulation of various forms of selenium in fish exposed to water of different synthetic matrices. It was anticipated that three distinct forms of selenium could be dissolved in the exposure water: selenate, selenite, and selenomethionine. Conventional methods of preparation and analysis of selenium did not allow for the determination of these three forms from a water sample. The methods designed and normally used for speciation of two of the compounds, i.e., selenate and selenite, will effectively convert some to all of any selenomethionine present to an inorganic form. Thus, it was necessary to design speciation methods which would selectively separate selenomethionine from the selenate/selenite species. In addition, there was no information in the literature as to the storage characteristics for selenomethionine either by itself or in combination with selenate/selenite species. Thus, a long-term storage study which investigated the variables of container material, water matrix, and temperature was necessary to validate the chosen method of preservation and storage for water samples collected from diluter exposure experiments.

Methods

Speciation techniques with Cu-Chelex: With wet chemical techniques (1, 2), selenium can be speciated into three classes or forms: selenate, selenite, and the organoselenium class, which includes both Se^0 and Se^{-2}. The chemical techniques employed were designed to fit the determinative approach, which was hydride generation atomic absorption spectrophotometry. This type of detection is only sensitive to the selenite form. When a water sample is analyzed "as is" (no prior preparation), the results will be indicative of the amount of selenite in solution. Typically, a second aliquant of water is then taken and subjected to a hot HCl reduction procedure, which reduces any selenate in the sample to selenite, giving selenate + selenite results. By performing a total selenium analysis (boiling with potassium persulfate to oxidize all selenium species to selenate, then hot HCl reduction to convert selenate to selenite) on yet a third aliquot of the water sample, the organoselenium class can be determined by difference (i.e., total minus selenate plus selenite). Since the hot HCl reduction procedure will destroy selenomethionine (convert selenomethionine to measurable selenite), water samples that contain this compound must be speciated in a different manner.

Free amino acids have classically been separated and characterized by passing the solution containing these free amines through a Cu-Chelex ligand exchange resin column (3-6). This exchange resin has not been characterized for selenomethionine. A 200-400 mesh Cu-Chelex resin material was produced which provided a superior ligand exchange and selenium capacity over the larger mesh sizes. Once produced, columns containing 5 mL bed volumnes of the resin were made and characterized as to optimum flow rate and chromatographic break point of selenomethionine. Experiments were conducted to assure that Se^{+6} and Se^{+4} did not interact with the Cu-Chelex material.

With quantitative selectivity of Cu-Chelex for selenomethionine, an overall speciation approach was developed for diluter exposure water. An aliquot of the water was passed through the Cu-Chelex column and the eluant collected. All of the selenomethionine was quantitatively retained by the Cu-Chelex, while inorganic forms of selenium (selenate and selenite) were quantitatively recovered in the collected eluant. The eluant was then split, with half being anlyzed by hydride generation atomic absorption to determine the selenite concentration. The other half of the eluant underwent hot HCl reduction to provide selenate and selenite results. The selenate concentration was then determined by difference. Another aliquot of water was then digested to determine the total selenium concentration.

From these above concentrations, the selenomethionine concentration was determined by difference, with the assumption that no additional unmeasured forms (species not transformed to selenite by these procedures) of selenium were present in the eluant. In practice, replicate water samples from the earliest and latest sampling times (using water from the highest exposure tanks) was treated with Cu-Chelex resin and the eluant divided into thirds. The first two portions (each 1/3 of the eluant) was either analyzed directly or treated with hot HCl reduction to determine the amounts of selenite and selenate in the eluant. The third portion of the eluant was analyzed for total selenium, and the difference was indicative of other unknown forms of selenium that may not be trapped by the Cu-Chelex column, or species not transformed to selenite by these procedures. Finally, a separate portion of the untreated water sample (not subjected to Cu-Chelex separation) was digested to determine the total selenium concentration in the untreated water. From all of the above concentrations, the selenomethionine was determined by difference.

Preservation experiments: Although there are considerable literature references for the perservation of selenate and selenite, there is little information regarding preservation of organoselenium compounds, either by themselves or in combination with selenate and selenite. Thus, the preservation behavior of three forms of selenium (selenate, selenite, and selenomethionine) under two sets of preservation conditions was examined. Eighteen bottles, nine constructed of conventional polyethylene and nine of Wheaton "400" brand borosilicate glass (all 500 mL size) were used in the experiment. All bottles were filled with 500 mL of equal concentrations of selenate, selenite, and selenomethionine at three different concentration levels: 10, 50, and 100 ppb. All solutions were acidified by making up in 1% HCl. The polyethylene bottles were stored at -20°C and the glass bottles at +4°C. All solutions were anlyzed at Day 0, 7, 30, 60, and 120. The selenomethionine standard was analyzed prior to Day 0 with an amino-acid analyzer to assure that this compound had not experienced breakdown.

Principal Findings

In an acidified (1% HCl) matrix alone, or in an acidified matrix with high ionic strength, waterborne selenate, selenite, and selenomethionine can be successfully preserved and stored for at least 4 months without any significant changes in concentration. Freezing at -20°C or refrigeration at +4°C is equally adequate.

Selenate, selenite, and selenomethionine can be successfully separated and quantified by the use of Cu-Chelex ligand exchange chromatography coupled with hydride generation atomic absorption spectrophotometry.

Research Needs

Selenomethionine was selected for fish exposure studies, both through water and diet, because it is readily available in a high purity state and produces similar fish growth and reproduction effects as found in fish from selenium contaminated environments. In addition, microcosm experiments have reported very high bioconcentration factors for selenium added as selenomethionine, suggesting that this species may contribute disproportionately to selenium bioaccumulation and toxicity in contaminated waters due to preferential uptake from water or transfer via food-chain organisms (6). Such research points to a need for additional study of the occurrence and toxicological importance of organoselenium compounds.

The chemical approaches used in this study, while fine for controlled laboratory situations, must be capable of being applied to selenium-contaminated environments. This research has shown that small Cu-Chelex columns can be used as preconcentration or contaminant-enrichment modules for amino-acid containing and/or proteinaceous organoselenium compounds that may occur at low concentrations in highly eutrophic selenium-enriched environments.

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